2004

Evaluation of injury and management strategies for stink bugs (Heteroptera: Pentatomidae) on cotton, Gossypium hirsutum L.

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EVALUATION OF INJURY AND MANAGEMENT STRATEGIES FOR STINK BUGS (HETEROPTERA: PENTATOMIDAE) ON COTTON, *GOSSYPIUM HIRSUTUM* L.

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by
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August, 2004

ACKNOWLEDGEMENTS

I wish to express my appreciation to the LSU AgCenter, the faculty and staff of the Department of Entomology, and the faculty and staff of the Macon Ridge Research Station. I would like to thank my major professor, Dr. Roger Leonard for his guidance, support, and council. Without a doubt, my research and professional successes have been a result of his leadership. I would also like to thank my minor professor, Dr. James Griffin, as well as other members of my graduate committee, Drs. Richard Story, Boyd Padgett, and Paul Wilson. The assistance provided by my fellow graduate students, Jeff Gore, Don Cook, Rhett Gable, Ashley Peters, Josh Temple, Kelly Tindall, and the numerous employees at the Macon Ridge Research Station, Mr. Ralph Sheppard, Mrs. Karla Emfinger, Mrs. Myra Purvis, and Mrs. Brandi Garber, are also appreciated. The funding provided for this project by Cotton Incorporated and the Louisiana Cotton Producers is appreciated. I also wish to thank my husband, Jonathan Siebert, and my parents, Tonnie and Jerry, for their support and understanding. Finally, I would like to express my appreciation to the LSU Ag Center for providing funding for my graduate school assistantship and to the Stanzel Family Foundation in Schulenburg, Texas, for their financial support of my educational endeavors during the last nine years.

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ABSTRACT

The effects of brown stink bug, *Euschistus servus* (Say), and southern green stink bug, *Nezara viridula* (L.), feeding on pre-flowering, flowering, and senescing cotton, *Gossypium hirsutum* L., plants were evaluated in field studies. Vegetative stage seedlings and flower buds (squares) were not significantly injured by adults or nymphs of either species in no-choice studies. Brown stink bug adults induced boll abscission, and reduced seedcotton yield and seed germination in bolls accumulating ≤ 350, ≤ 550, and 101 to ≤ 600 heat units beyond anthesis, respectively. In free-choice tests, boll preference was evaluated during each of the initial five weeks of flowering. Boll density increased from 5.1 to 6.6-fold from week one to week five. There was a corresponding 4.6 to 6.2-fold increase in total bolls injured. Boll injury ranged from 10.7% (week 4) to 27.4% (week 2) and 9.2% (week 3) to 16.0% (week 2) in 2002 and 2003, respectively. The frequency of injured bolls was highest for bolls accumulating 165.2 through 672 heat units beyond anthesis (1.161 to 3.586 cm diameter). However, brown stink bug significantly reduced seedcotton yields during weeks four and five due to the inability of cotton plants to compensate for injured bolls. Infestations of southern green stink bug during boll maturation, in combination with persistent rainfall and humidity, increased the proportion of rotted (2.0-fold) and "hard locked" (1.4-fold) bolls compared to a non-infested treatment. Although stink bug injury was observed in hard locked (35.8%) and harvestable (20.3%) bolls, other abiotic and/or biotic factors are contributing to late-season harvest losses. In laboratory and field studies, the order of susceptibility (least to most) of stink bug species and life stages to insecticides commonly used for management was adult *Euschistus* spp. < late-instar nymphs < southern green stink bug adults. These studies defined brown stink bug and southern green stink

bug to be significant pests of cotton during boll development stages. Stink bug management strategies should consider species, life stages, and the characteristics of specific insecticides.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Cotton Insect Pest Management

The development of integrated pest management (IPM) programs for agriculture has significantly changed crop production systems in the United States. IPM has been regarded as the single most important event in crop protection science during the last three decades (Pedigo and Higley 1992). The origins of IPM were centered on the misuse and overdependence with chemical pesticides. Prior to the implementation of IPM, the indiscriminant use of pesticides resulted in pesticide-resistant insect populations, rapid resurgence of target pest populations following treatment, outbreaks of secondary pests, and undesirable environmental effects (Smith et al. 1976).

Cotton, *Gossypium hirsutum* L., production in the United States changed dramatically with the implementation of IPM. Recent technologies adapted by producers have further improved IPM in production systems. A general reduction in the frequency of broad-spectrum insecticide applications against cotton pests has occurred as a result of boll weevil, *Anthonomus grandis grandis* Boheman, eradication, the commercialization of target-selective insecticides, and introduction of Bollgard® cotton cultivars.

Prior to the entry of the boll weevil to Texas from Mexico in 1892, cotton production was rarely affected by biotic pests; however, since 1909 direct losses of cotton fiber and seed have exceeded more than $200 million annually (Smith 1995, Leonard et al. 1999). The first successful attempt to eradicate the boll weevil began in the late 1970's in North Carolina and Virginia (Brazzell et al. 1996). By 1993, the boll weevil was considered to be eradicated in California, Arizona, Virginia, North Carolina, South Carolina and contained with maintenance

programs in Georgia, Alabama, and Florida. Insecticide use (total lb AI/acre) on cotton was reduced in those areas by greater than 50% compared to use before eradication (Brazzel et al. 1996). Louisiana is one of several states currently in an active eradication zone (El-Lissy and Grefenstette 2001).

Current pesticide regulatory policy has driven crop protection companies to develop "reduced risk pesticides" that are used at low application rates with few environmental hazards (Osteen and Padgitt 2002). Insecticides that exhibit selective toxicity to arthropods and exploit unique target sites include the insect growth regulators (chitin synthesis inhibitors, ecdysone agonists, and juvenile hormone analogs), avermectins (emamectin benzoate), spinosyns derived from the soil actinomycete *Saccharopolyspora spinosa* (spinosad), neonicotinoids (imidacloprid, thiamethoxam, acetamiprid, clothianidin, thiacloprid), and oxadiazines (indoxacarb). Many of these new insecticide chemistries are remarkably sensitivity to lepidopterous insects and are also less toxic toward sucking insects and coleopterans (Thompson et al. 1996, Holloway et al. 1999, Wing et al. 2000). Additionally, selective insecticides can preserve beneficial arthropods in both conventional and Bollgard cotton cultivars (Holloway et al. 1999).

Bollgard cotton cultivars, that express the Cry IA(c) protein from *Bacillus thuringiensis* Berliner var. *kurstaki* are active only against selected lepidopterous insects (Gould 1998). This includes the most injurious caterpillar pest on cotton, the tobacco budworm, *Heliothis virescens* (F.) (Hardee et al. 2001). Supplemental controls have been required for bollworm, *Helicoverpa zea* (Boddie); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); beet armyworm, *Spodoptera exigua* (Hübner); soybean looper, *Pseudoplusia includens* (Walker); and non-lepidopterous pests [plant bugs (Hemiptera: Miridae) and stink bugs (Heteroptera: Pentatomidae)] due to the limited spectrum of activity for Bollgard cotton. Insecticide applications have been reduced to fewer

than 3 applications per year in some states (Turnipseed et al. 2001).

Pest Status of Stink Bugs

An increase in abundance of secondary pests such as stink bugs and plant bugs has occurred in cotton across the mid-southern and southeastern United States (Greene and Herzog 1999, Leonard et al. 1999, Roberts 1999, Bachelor and Mott 2000, Roof and Arnette 2000). Historically, stink bugs have been an occasional pest because infestations were indirectly removed with insecticide applications directed for the boll weevil and late-season heliothine infestations (McPherson and McPherson 2000). Based upon the standards of the previous decade, cotton insect pest management is characterized by low insecticide use (Greene et al. 1998, Roof and Arnette 2000, Leonard and Emfinger 2002). In North Carolina, a mean of 0.75 applications from 1996 to 1999 in Bollgard cotton, has been associated with a 4-fold increase in the level of stink bug damaged bolls when compared with conventional insecticide-treated cotton fields (Bachelor and Mott 2000).

Further reductions in insecticide use should continue upon release of Bollgard II® cotton cultivars that express both Cry 1A(c) and Cry 2A(b) protein. The Cry2A(b) protein demonstrates better control of bollworm, soybean looper, beat armyworm, and fall armyworm compared to that of Bollgard cotton (Stewart et al. 2001). Other cotton cultivars with similar traits (WideStrike®, VipCot®) and spectrum of activity are also expected to be released (Adamczyk et al. 2003, Huckaba et al. 2003, Mascarenhas et al. 2003). Therefore, even fewer foliar insecticide applications will be required for management of lepidopteran pests. It is likely that the status of secondary pests will escalate in cotton production systems utilizing transgenic cotton cultivars that target lepidopteran pests.

Stink bugs were included in cotton loss estimates for each state beginning in 1993

(Williams 1994). Louisiana reported infested acreage initially during 1995 (0.8%) (Williams 1996). Stink bugs infested 5,294,862 acres across the seventeen states of the cotton belt in 2000, ranking fifth among all arthropod pests (Williams 2001). In Louisiana, the number of acres infested with stink bugs in 1995 through 2002 has risen from 8,367 to 363,200, respectively (Williams 1996, 2003). Of the total acreage planted to cotton in Louisiana, 73.8% was infested with stink bugs in 2002 (Williams 2003).

Stink Bug Complex in Louisiana

The common pest species found in row crops include brown stink bug, *Euschistus servus* (Say); *Euschistus quadrator* Rolston; *Euschistus tristigmus* (Say); the southern green stink bug, *Nezara viridula* (L.); and the green stink bug, *Acrosternum hilare* (Say) (McPherson et al. 1979a, McPherson et al. 1979b). The impact of southern green stink bug on Louisiana field crops is much more severe compared to the other species, and it is considered the primary stink bug pest of most crops in the southern United States (McPherson et al. 1994). In recent years, however, brown stink bug has been encountered more often than in previous reports. Little research have been directed toward the species in this genus (Boethel 2000).

An early biological description of brown stink bug indicated a state-wide distribution in Louisiana, but not in sufficient numbers to be listed as a destructive insect (Parker 1941). Several biological traits of brown stink bug may explain an increase in incidence during recent years. These attributes may potentially influence pest status compared to southern green stink bug. Brown stink bug is less susceptible to many insecticides (Emfinger et al. 2001, Fitzpatrick et al. 2001a). The supercooling point (a statistic for representing the lower limit of survival of freezing-intolerant species) for brown stink bug adults is lower (-15°C) than that for southern green stink bug adults (-11°C) (Elsey 1993). Therefore, brown stink bug may withstand extreme

winter temperatures compared to southern green stink bug. Additionally, brown stink bug maintains a wider host range (Jones and Sullivan 1981, 1982).

Stink Bug Biology

Phytophagous stink bugs are economically important pests complex of grain, fruit, and fiber crops (Panizzi 1997, McPherson and McPherson 2000). Stink bug feeding and development has been observed on approximately 252 plants, of which the following crops are included: alfalfa, *Medicago sativa* L.; cowpea, *Vigna unguiculata* (L.) Walper; corn, *Zea mays* L.; cotton; macadamia, *Macadamia integrifolia* Maiden & Betche; rice, *Oryza sativa* L.; sorghum, *Sorghum bicolor* (L.) Moench; soybean, *Glycine max* (L.) Merrill; tomato, *Lycopersicon esculentum* Miller; wheat, *Triticum aestivum* (L.); and various fruit crops (McPherson and McPherson 2000). The biology, life history, and population dynamics of stink bugs have been studied in numerous geographical locations including Arkansas, California, Florida, Georgia, Illinois, Louisiana, and South Carolina (Drake 1920, Parker 1941, Rolston and Kendrick 1961, Harris and Todd 1980, Jones and Sullivan 1981, Munyaneza and McPherson 1994, Ehler 2000).

Stink bug eggs are deposited on host plants in polygonal clusters (Todd 1989). Each cluster may contain several to greater than 70 barrel-shaped eggs that are tightly packed in rows (Esselbaugh 1946, Bundy and McPherson 2000a). First instar nymphs eclose within ca. 5 d and remain aggregated on or near the egg cluster without feeding (Lockwood and Story 1986, Todd 1989). Subsequent instars disperse slightly and begin feeding; however, aggregation of individuals from the same egg cohort may occur through the final instar (Todd and Herzog 1980). Stink bugs develop through five nymphal instars (Decoursey and Esselbaugh 1962, Todd 1989). The duration of immature development may range from ca. three to five weeks,

depending on temperature (Todd 1989). Adult and nymphal stages of stink bugs generally aquire food by puncturing plant tissue with their piercing sucking mouthparts and removing the cell's contents (McPherson et al. 1994, Panizzi 1997). Stink bugs feed upon numerous plant parts including stems, petioles, flowers, fruits, and seeds (Chandler 1955, Clower 1958, Townsend and Sedlacek 1986, Russin et al. 1988, Apriyanto et al. 1989).

Typically, stink bugs overwinter in the adult stage in a reproductive diapause beneath leaf litter, bark, wood piles, and within other objects that offer protection from environmental extremes (Todd 1976, Todd and Herzog 1980, McPherson et al. 1994). Jones and Sullivan (1981) reported winter mortality levels and spring emergence patterns among several hemipterans from various overwintering habitats. Brown stink bug was the most commonly trapped hemipteran species (representing 22% of the 522 individuals among 47 species) from cone traps during spring, that were originally placed in six ground habitats during late winter. In a second study, cages were placed over wild radish, *Raphanus raphanistrum* L., deciduous leaf litter, and soybean stubble and infested with southern green stink bug adults. No southern green stink bug survived the winter (dead individuals were found upon inspection); however, three brown stink bugs emerged from those cages placed over soybeans the previous fall.

Adult stink bugs become active in the spring, and have been observed as early as March in Arkansas (Rolston and Kendrick 1961). Generally, the first generation of stink bugs in the southeastern United States can be found in clovers (*Vicia* spp., *Trifolium* spp.), early vegetables [mustard, *Brassica* spp.; turnip, *Brassica napus* L.; beet, *Beta* spp.; radish, *Raphanus* spp.], small grains, field corn, and weed hosts (showy crotalaria, *Crotalaria spectabilis* Roth; coffee senna, *Cassia occidentalis* L.) (Todd 1976, Todd and Herzog 1980, McPherson et al. 1994). As the season progresses, subsequent generations of stink bugs migrate to cultivated hosts, with field

corn and soybean suggested to be common hosts (Todd 1976). Stink bug movement from wild host plants to cultivated field crops coincides with seed development stages of the hosts (Rolston and Kendrick 1961, Todd and Herzog 1980). As spring plant hosts senesce and become unattractive for feeding and oviposition, adults immigrate to hosts that are more acceptable for nutrition and reproduction (Todd and Herzog 1980, Panizzi and Meneguim 1989).

Population Dynamics of Stink Bugs in Agroecosystems

In Louisiana, the agricultural landscape provides suitable cultivated and non-cultivated hosts, both temporally and spatially, for utilization by stink bugs. Boethel et al. (1986) studied the impact of southern green stink bug infestations in corn and grain sorghum on infestations in soybean. Southern green stink bug were observed on corn until shortly before harvest maturity; however, infestations began declining as grain began to mature (brown stink bugs were rarely found). The population decline in field corn corresponded with increasing densities on grain sorghum and maturity group IV soybeans, with densities greater on the latter. Frequently, stink bug populations exceeded the treatment threshold in soybean fields adjacent to corn and grain sorghum fields (Boethel et al. 1986).

The dispersal of stink bugs among crops has been studied in cotton-soybean agroecosystems. In Georgia, two soybean varieties representing a maturity group V and VII were planted adjacent to conventional and Bollgard cotton cultivars. Stink bugs preferred soybeans over cotton (Bundy and McPherson 2000b). Stink bugs were observed in both soybean varieties at the initiation of pod formation (R3 growth), with migration into the maturity group VII variety once the maturity group V variety reached maturity (R7 growth stage) (Fehr et al. 1971). No significant numbers of stink bugs were observed in cotton when soybean were available. However, peak numbers in cotton occurred during the time when all stages of boll

development were present. The authors concluded that soybean could be used as a trap crop to reduce stink bug numbers on cotton.

Southern green stink bug was the most common species encountered in both of the previously described studies of stink bug dispersal among crop hosts. Currently, it is unclear why brown stink bug has historically been of little economic importance on soybean (McPherson and McPherson 2000). Most explanations have centered on the wide host range of brown stink bug. Jones and Sullivan (1982) sampled 26 species from April through November and detected brown stink bug on 14 plants, while southern green stink bug was detected on four. The authors concluded that large populations of brown stink bug may not develop on soybean when non-cultivated plant hosts, of preferred phenological stages for stink bugs, are available both temporally and spatially. Therefore, the availability of alternate hosts in an agroecosytem may enhance the annual population growth of brown stink bug, to a greater extent than southern green stink bug.

The ability of brown stink bug to utilize a wide range of hosts, with less dependence on soybean for growth and reproduction, may have an impact in Louisiana where the Conservation Reserve Program (CRP) has been embraced by many landowners. CRP was established in 1985 with goals of reducing erosion, improving water quality, and enhancing wildlife habitats. In Louisiana, the parishes of greatest cotton production are also the same parishes in which large amounts of acreage are devoted to CRP. In Louisiana, the total acreage planted to cotton annually occurs in five parishes (Tensas, Morehouse, Franklin, Richland, and Madison), which accounts for 26.3% of the total CRP acres in the state (Anonymous 2001b, USDA 2001). CRP land that is abundant in wild host plants may be impacting brown stink bug population densities.

Cotton could potentially become a more frequent host for stink bugs in Louisiana due to

the decline in number of acres planted to soybean. Since 1990, soybean acreage has decreased from approximately 1,800,000 to 700,000 in 2001 (Anonymous 2001a). In Australia, the concentration of stink bugs on reproductive stage soybean for feeding and oviposition is not caused by the attractiveness of the plant, but the non-availability of other suitable hosts (Velasco and Walter 1992). Therefore, widespread availability of cotton and field corn in Louisiana may increase stink bug infestations on those crops, as soybean acreage declines. Stink bugs may be present in corn fields from seedling emergence through ear formation and grain development. Feeding by stink bugs on corn seedlings, corn ears prior to emergence from the stalk (V15 growth stage), and mature ears result in the production of tillers, malformed (curved) ears, and loss of individual kernels, respectively (Clower 1957, Negron and Riley 1987, Smith 1990).

Stink Bug Injury to Cotton

Much of the data collected on stink bugs in cotton is in relation to their occurrence on bolls. Little data is available on the impact of stink bugs on cotton seedlings, or on flower buds (squares). Cotton seedlings and squares are particularly sensitive to injury from other bugs, including clouded plant bug, *Neurocolpus nubilus* (Say), and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Tugwell et al. 1976). Tarnished plant bug can feed in the plant terminal of cotton seedlings; subsequently, causing the terminal to abort (Tugwell et al. 1976, Burris et al. 1997). Feeding damage by tarnished plant bug also results in the abscission of pin-head squares, necrosis of anthers and staminal columns, and crinkling and cupping of flower petals (Burris et al. 1997).

Injury to cotton during fruiting stages has been investigated for several stink bug species. Abscission of small bolls, caused by *Euschistus impectiventris* Stål, *Chlorochroa sayi* Stål, and *A. hilare* feeding, have been reported (Wene and Sheets 1964, Barbour et al. 1990). In cotton,

abscission of young bolls can be a natural occurrence and is accentuated by low light intensity, extreme temperatures, and water stress (Oosterhuis and Jernstedt 1999). Stink bug injured bolls may only be abscised when the plant is under these additional stresses (Barbour et al. 1990). Abscission of young bolls is also characteristic of feeding by tarnished plant bug (Burris et al. 1997, Russell 1999). Roach (1988) caged entire plants for a continuous two month period during the cotton fruiting period and determined the number of abscised fruiting structures (squares and bolls) was not correlated with the presence of green stink bug and brown stink bug.

In cotton, flowers do not abscise and only bolls less than 10 days old abscise (Jones 1963). Older bolls that are injured remain on the plant but may display feeding symptoms on the exocarp, endocarp, seed, or lint. External injury to bolls may be described as dark, circular indentations. Dark feeding punctures or wart-like growths (callous tissue) may be found on the internal carpel wall (endocarp) (Wene and Sheets 1964, Greene and Herzog 1999, Bundy et al. 2000). Bundy et al. (2000) studied the type of injury caused by a combination of brown stink bug, green stink bug, and southern green stink bug, and the time for symptoms to become visible. Internal warts and external marks are formed within 48 h (Bundy et al. 2000). However, external symptoms were found to be an inaccurate estimate of internal boll injury because approximately 20% of injured bolls with internal warts lacked external symptoms of feeding (Bundy et al. 2000). Diagnosing stink bugs as the primary causal agent of boll abscission and injury is problematic because plant bug species cause similar effects. Stink bugs and plant bugs both cause young bolls to abscise (Wene and Sheets 1964, Burris et al. 1997). For older bolls, internal warts formed by tarnished plant bug are indistinguishable from that of fourth instar southern green stink bug (Greene et al. 1999) and probably for other stink bug stages. Southern green stink bug creates significantly more internal warts per boll than tarnished plant bug

(Greene et al. 1999). In contrast, Wene and Sheets (1964) reported feeding by plant bugs on more mature bolls does not cause warts on the endocarp.

Boll age classes that are susceptible to injury by stink bugs have been investigated in several studies. Fifth instar southern green stink bug confined to a 9-d old boll for 3.5 d resulted in 1.4-fold and 1.4 to 3.6-fold more warts per boll than southern green stink bug adults or earlier instars, respectively (Greene et al. 1999). Additionally, as boll age increased, damage by fifth instar nymphs decreased from 4 to 21 d after anthesis (Greene et al. 1999, Greene et al. 2001a). Southern green stink bug and green stink bug adults or late instar nymphs caged on fruiting branches containing a first (0-3 d beyond anthesis), second (6-9 d beyond anthesis), and third (12-15 d beyond anthesis) position boll reduced seedcotton yield (Lee et al. 1999). Infestations of brown stink bug on bolls 11 and 14 d beyond anthesis have also significantly reduced seedcotton yield (Fromme 2000).

Yeargan (1977) determined that quantitative and qualitative damage to soybean caused by green stink bug is comparable to southern green stink bug. Based on these results, injury among species is assumed to be similar for cotton (Bundy et al. 2000). However, in pecan, *Carya illinoensis* (Wangenh) K. Koch, brown stink bug caused 73% fruit drop when feeding prior to shell hardening whereas southern green stink bug caused less than 53% (Dutcher and Todd 1983).

Bolls damaged from stink bugs may be exhibited in other ways. At harvest, fields that have sustained stink bug infestations have been associated with “hard locked” bolls (Wene and Sheets 1964, Barbour et al. 1990, Turnipseed et al. 1995). “Hard locked” bolls are described as bolls that crack and partly open but fail to fluff (Halloin 1986). The incidence of “hard locked” bolls has ranged from 12 to 41% in cotton fields infested with stink bugs (Turnipseed et al.

1995). Bolls exhibiting hard lock are difficult to harvest with mechanical pickers (Halloin 1986, Leonard et al. 1999). Additionally, bolls injured by insects may provide an entrance wound for bacterial and fungal pathogens that cause boll rot (Kirkpatrick and Rothrock 2001).

Lint and seed quality may also be affected in cotton bolls that do not exhibit the "hard locked" condition. Penetration of older bolls, by stink bugs can result in discolored, yellowed lint (Wene and Sheets 1964, Leonard et al. 1999). Lower germination rates have been reported for seed in bolls previously punctured by green stink bug, *C. uhleri*, and *Euschistus conspersus* Uhler (Toscano and Stern 1976, Barbour et al. 1990).

Sampling Stink Bugs in Cotton

Estimating stink bug densities and injury levels is extremely difficult in cotton (Greene and Herzog 1999). The shake sheet (drop cloth) provides the best available and practical means of detecting stink bugs in a row crop. Examining bolls for stink bug feeding is also an effective monitoring tool (Greene and Herzog 2000). Thresholds for initiating insecticide applications against stink bugs in cotton have been established using these sampling methods in the mid-southern and southeastern United States (Table 1.1).

Table 1.1. Recommended timing of application for control of stink bugs in mid-southern and southeastern cotton producing states.

State	Timing of Application	Reference
Alabama	1 stink bug/20 row-feet or 10% of small bolls (1/3 size) display damage	Anonymous (2002)
Arkansas	1 stink bug/6 row-feet or 20% of medium sized bolls display internal signs of feeding and stink bugs are present	Johnson et al. (2002)
Florida	4 stink bugs/100 sweeps or 1 stink bug/6 row-feet	Sprenkel (2002)

Table 1.1. Continued.

State	Timing of Application	Reference
Georgia	1 stink bug/6 row-feet or 20% of quarter-sized bolls display internal signs of stink bug feeding and stink bugs are observed in the field	Roberts et al. (2003)
Louisiana	1 stink bug adult or nymph/6 row feet, 5 adults or nymphs/100 sweeps, or 20% internal injury in 12 to 16 d old bolls	Bagwell et al. (2002)
Mississippi	5 stink bug adults or nymphs (1/4 inch or greater) /100 plants or 1 bug/6 row-feet (1/4 inch or greater)	Layton (2002)
North Carolina	1 stink bug adult or large nymph/6 row-feet or 1adult or large nymph/25 sweeps or 20 stink bug damaged (internal feeding) thumb-sized bolls/100	Bachelor and Van Duyn (2003)
South Carolina	1 stink bug adult or large nymph/6 row-feet or 20% boll damage in quarter-sized bolls	Roof and Arnette (2000)
Tennessee	1 stink bug/6 row-feet	Patrick and Lentz (2001)
Virginia	1 stink bug/25 sweeps or 10% damaged quarter-sized bolls	Herbert and Chappell (2003)

Stink Bug Susceptibility to Insecticides

Initiating control measures against stink bugs in cotton requires more than detecting the pest and estimating infestation levels. Proper identification of species and developmental stages is necessary because of variation in insecticide susceptibility among species and life stages. McPherson et al. (1979a) demonstrated in laboratory studies with adults that *Edessa bifida* (Say) had a significantly higher LD_{50} than that for other stink bug species [green stink bug, brown stink bug, *E. tristigmus*, southern green stink bug, *Podisus maculiventris* (Say), *Thyanta pallidovirens* Stäl] when exposed to methyl parathion. The LD_{50}'s for fifth instar southern green stink bug, green stink bug, and brown stink bug also were higher than for their corresponding adults. In laboratory bioassays, Greene et al. (2001b) did not observe differences in insecticide

susceptibility between adults and fifth instar nymphs within a species (southern green stink bug and brown stink bug). However the pyrethroids (*zeta*-cypermethrin, cypermethrin, and cyfluthrin), with the exception of bifenthrin, provided mortality of brown stink bug that was lower compared to that of southern green stink bug.

Insecticide recommendations in Louisiana for soybean and cotton were separated for *Euschistus* spp. and southern green stink bug/green stink bug in 2001 (Bagwell et al. 2001, Baldwin et al. 2001). Products registered for use in cotton that are recommended by the Louisiana Cooperative Extension Service for control of southern green stink bug include the organophosphates (acephate, dicrotophos, and methyl parathion) and the pyrethroids (cyfluthrin, bifenthrin, *zeta*-cypermethrin, deltamethrin, *lambda*-cyhalothrin, and tralomethrin) (Bagwell et al. 2001). Pyrethroid insecticides currently are not recommended for control of brown stink bug in Louisiana.

Acephate (Orthene 75SG or 97SP) applied at rates of 0.75, 0.80, or 1.0 lb AI/acre have significantly reduced southern green stink bug and brown stink bug on soybean compared to that in non-treated areas (Crowe et al. 2000, Willrich et al. 2000, Fitzpatrick et al. 2001a,b). Brown stink bug, southern green stink bug, and green stink bug adults and nymphs have been successfully controlled with dicrotophos (Bidrin 8EC) applied at 0.375 and 0.5 lb AI/acre (Bachelor and Mott 2002, Fitzpatrick et al. 2002a,b). Brown stink bug and southern green stink bug adults and nymphs have also been reduced with methyl parathion (4EC) at 0.5 and 1.0 lb AI/acre (Willrich et al. 2000, Fitzpatrick et al. 2002a).

Generally, pyrethroids have not provided effective control of brown stink bug at those rates commonly used in cotton. Brown stink bug adults in soybean were not significantly reduced five days after treatment with *lambda*-cyhalothrin (0.025 lb AI/acre); however, those

applications did significantly reduce numbers of brown stink bug nymphs (Fitzpatrick et al. 2001b). Bifenthrin, however, applied at 0.05 and 0.07 lb AI/acre (within the labeled use rate for cotton) controlled brown stink bug comparable to southern green stink bug (Emfinger et al. 2001). Topical applications of bifenthrin to brown stink bug adults and nymphs resulted in 65 and 67% mortality, respectively (Greene et al. 2001b). Mortality of southern green stink bug adults and nymphs exposed to bifenthrin was 82 and 74%, respectively.

Resistance Monitoring

Monitoring for changes in susceptibility to insecticides is important because of the increasing importance of this pest in cotton. The adult vial test (AVT) has become a popular method for monitoring changes in insecticide susceptibility. The AVT was initially developed by Plapp et al. (1987) for tobacco budworm adults, and has since been modified for adults and/or larvae of several insects including tobacco budworm (Campanhola and Plapp 1989), greenbug, *Schizaphis graminum* (Rondani) (Archer et al. 1994), boll weevil (Kanga et al. 1995), soybean looper (Mink et al. 1993), and tarnished plant bug (Snodgrass 1996). The AVT is an attractive monitoring tool because it requires inexpensive equipment, offers rapid results, and can be used by producers, crop consultants, and researchers (Plapp et al. 1987, Snodgrass 1996).

Preliminary studies compared the susceptibility of stink bugs to insecticides using the AVT (Emfinger et al. 2001). These results indicated differences in pyrethroid susceptibility between brown stink bug and southern green stink bug adults to pyrethroid insecticides. The LC_{50}'s for brown stink bug exposed to cyfluthrin, cypermethrin, and λ-cyhalothrin were 3.9, 2.9, and 4.3-fold higher, respectively, than southern green stink bug. The LC_{50}'s of brown stink bug and southern green stink bug exposed to bifenthrin were not significantly different.

Although previous research has examined the effect of stink bugs on cotton plants,

southern green stink bug and green stink bug have received the most attention. With increased occurrence of brown stink bug in Louisiana and other mid-southern and southeastern cotton producing states, further data on this particular species must be obtained. Additionally, specific questions related to the interactions among stink bugs, crop phenology, boll injury, and yield losses are needed to refine current sampling protocols that rely on boll injury to initiate control measures.

Objectives

1. To evaluate injury to pre-flowering and flowering cotton by brown stink bug and southern green stink bug.
2. To investigate boll injury and yield losses associated with brown stink bug during flowering.
3. To define cotton boll age cohorts injured by brown stink bug during flowering.
4. To determine the influence of southern green stink bug on late-season yield losses in cotton.
5. To evaluate insecticide toxicity against stink bug species and developmental stages using laboratory and field techniques.

References Cited

Adamczyk, J.J., J. Gore, and J. Pellow. 2003. Evaluation of Dow AgroSciences' cry1ac/cry1f trait for improved lepidopteran control, pp. 1567-1571. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Anonymous. 2001a. Soybean producing parishes. http://www.agctr.lsu.edu/cotton/soyparis.html.

Anonymous. 2001b. USDA-NASS Published Estimates Data Base. http://www.nass.usda.gov:81/iped/top.htm.

Anonymous. 2002. Cotton insect, disease, nematode, and weed control recommendations for 2002. Alabama Cooperative Extension Service. http://www.aces.edu/dept/extcomm/publications/anr/2002IPM/COTTON.02.PDF.

Apriyanto, D., L.H. Townsend, and J.D. Sedlacek. 1989. Yield reduction from feeding by *Euschistus servus* and *Euschistus variolarius* (Heteroptera: Pentatomidae) in stage V2 field corn. J. Econ. Entomol. 82: 445-448.

Archer, T.L., E.D. Bynum, Jr., and F.W. Plapp, Jr. 1994. Chlorpyrifos resistance in greenbugs (Homoptera: Aphididae): cross-resistance and synergism. J. Econ. Entomol. 87: 1437-1440.

Bachelor, J.S. and D.W. Mott. 2000. Small plot and large-scale comparison of stink bug damage to bollgard and conventional cotton in North Carolina, pp. 1117-1119. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bachelor, J.S. and J.W. Van Duyn. 2003. 2003 North Carolina agricultural chemical manual: insect control on cotton. http://ipm.ncsu.edu/agchem/chptr5/505.pdf.

Bagwell, R.D., J.W. Barnett, B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2001. Cotton insect control, 2001. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Bagwell, R.D., Stewart, S., B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2002. Cotton insect control, 2002. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Baldwin, J.L., D.J. Boethel, and R. Leonard. **2001.** Control soybean insects 2001. Louisiana Cooperative Extension Service. Pub. No. 2211. 13 pp.

Barbour, K.S., J.R. Bradley, and J.S. Bacheler. 1990. Reduction in yield and quality of cotton damaged by green stink bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 83: 842-845.

Boethel, D.J. 2000. Foreword. *In* J.E. McPherson and R.M. McPherson [eds.], Stink bugs of economic importance in America north of Mexico. CRC Press, Boca Raton, FL.

Boethel, D.J., J.S. Russin, M.B. Layton, A. Sparks, and D.B. Orr. 1986. Annual report to the Louisiana soybean and grain research and promotion board, 1986. Louisiana State University Agricultural Center, Baton Rouge, LA.

Brazzel, J.R., J.W. Smith, and E.F. Knipling. 1996. Boll weevil eradication, pp. 629-654. *In* E.G. King, J.R. Phillips, and R.J. Coleman [eds.], Cotton insects and mites: characterization and management. The Cotton Foundation, Memphis, TN.

Bundy, C.S. and R.M. McPherson. 2000a. Morphological examination of stink bug (Heteroptera: Pentatomidae) eggs on cotton and soybean, with a key to genera. Ann. Entomol. Soc. Am. 93: 616-624.

2000b. Dynamics and seasonal abundance of stink bugs (Heteroptera: Pentatomidae) in a cotton-soybean ecosystem. J. Econ. Entomol. 93: 697-706.

Bundy, C.S., R.M. McPherson, and G.A. Herzog. 2000. An examination of the external and internal signs of cotton boll damage by stink bugs (Heteroptera: Pentatomidae). J. Entomol. Sci. 35: 402-410**.**

Burris, E., J.H. Pankey, B.R. Leonard, and J.B. Graves. **1997.** Tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), in cotton. Research Information Sheet 101. Louisiana State University Agricultural Center. 6 pp.

Campanhola, C. and F.W. Plapp, Jr. 1989. Pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae): insecticide bioassays and field monitoring. J. Econ. Entomol. 82: 22-28.

Chandler, S.C. 1955. Biological studies of peach catfacing insects in Illinois. J. Econ. Entomol. 48: 473-475.

Clower, D.F. 1958. Damage to corn by the southern green stink bug. J. Econ. Entomol. 51: 471-473.

Crowe, B.D., R.M. McPherson, and M.L. Wells. **2000.** Control of stink bugs, velvetbean caterpillar and threecornered alfalfa hoppers on Georgia soybeans, 1999. Arthropod Management Tests 25: 305-306.

Decoursey, R.M. and C.O. Esselbaugh. 1962. Description of the nymphal stages of some North American Pentatomidae. Ann. Entomol. Soc. Am. 55: 323-342.

Drake, C.J. 1920. The southern green stink bug in Florida. Florida State Plant Board Quarterly Bull. 4: 41-94.

Dutcher, J.D. and J.W. Todd. 1983. Hemipteran kernel damage of pecans, pp. 1-10. *In* J.A. Payne [ed.], Pecan pest management-are we there? Misc. Publ. Entomol. Soc. Am. 13: 1-140.

Ehler, L.E. 2000. Farmscape ecology of stink bugs in northern California. Entomological Society of America, Lanham, MD. 59 pp.

El-Lissy, O. and B. Grefenstette. 2001. Boll weevil eradication-national status, 2000, pp. 776-781. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Elsey, K.D. 1993. Cold tolerance of the southern green stink bug (Heteroptera: Pentatomidae). Environ. Entomol. 22: 567-570.

Emfinger, K., B.R. Leonard, J. Gore, and D. Cook. 2001. Insecticide toxicity to southern green, *Nezara viridula* (L.), and brown, *Euschistus servus* (Say), stink bugs, pp. 1159-1161. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Esselbaugh, C.O. 1946. A study of the eggs of the pentatomidae. Ann. Entomol. Soc. Am. 39: 667-691.

Fehr, W.R, C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybean, *Glycine max* (L.). Crop Sci. 1: 929-931.

Fitzpatrick, B.J., M.E. Baur, and D.J. Boethel. 2001a. Evaluation of insecticides against stink bug on soybean, 2000. Arthropod Management Tests 26: F95. http://www.entsoc.org/Protected/AMT/AMT26/INDEX/ASP.

Fitzpatrick, B.J., M.E. Baur, and D.J. Boethel. **2002a.** Evaluation of insecticides against the brown stink bug, 2001. Arthropod Management Tests 27: F93. http://www.entsoc.org/ Protected/AMT/AMT27/INDEX.ASP.

Fitzpatrick, B.J., J. Gore, M.M. Willrich, and D.J. Boethel. **2002b.** Evaluation of insecticides against stink bugs, 2001. Arthropod Management Tests 27: F99. http://www.entsoc.org/Protected/AMT/AMT27/INDEX/ASP.

Fitzpatrick, B.J., M.M. Willrich, D.J. Boethel, and B.R. Leonard. **2001b.** Evaluation of insecticides against stink bug on soybean, 2000. Arthropod Management Tests 26: F96. http://www.entsoc.org/Protected/AMT/AMT26/INDEX.ASP.

Fromme, D.D. 2000. The effect of boll age on stink bug feeding, boll damage and yield loss, p. 10. Cotton Incorporated Research Summary, Raleigh, NC.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701-726.

Greene, J.K. and G.A. Herzog. 1999. Management of stink bugs using symptoms of boll injury as a monitoring tool, pp. 1041-1044. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K. and G.A. Herzog. **2000.** Monitoring for and management of stink bugs, pp. 1120-1121. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001a. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council. Memphis, TN.

Greene, J.K., S.G. Turnipseed, and M.J. Sullivan. **1998.** Managing stink bugs in Bt cotton, pp. 1174-1177. *In* P. Dugger and D. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. **1999.** Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and O.L. May. 2001b. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403-409.

Halloin, J.M. 1986. Weathering: changes in planting seed quality between ripening and harvest, pp. 475-481. *In* J.R. Mauney and J. McD. Stewart [eds.], Cotton physiology. The Cotton Foundation, Memphis, TN.

Hardee, D.D., J.W. Van Duyn, M.B. Layton, and R.D. Bagwell. 2001. *Bt* cotton and management of the tobacco budworm-bollworm complex. USDA-ARS Pub No. 154.

Harris, V.E. and J.W. Todd. 1980. Comparative fecundity, egg fertility, and hatch among wild-type and three laboratory-reared generations of the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). J. Entomol. Sci. 15: 245-252.

Herbert, D.A. and G.F. Chappell. 2003. 2003 Virginia cotton insect control. Virginia Cooperative Extension. http://www.ext.vt.edu/pubs/pmg/fc4d.pdf.

Holloway, J.W., N.W. Forrester, and B.R. Leonard. 1999. New insecticide chemistry for cotton IPM. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Huckaba, R.M., R.B. Lassiter, X. Huang, C.A. Blanco, V.B. Langston, L.B. Braxton, F.J. Haile, J.M. Richardson, and J. Pellow. 2003. Field efficacy of Dow-AgroSciences MXB-13 transgenic cotton for control of cotton bollworm and tobacco budworm, pp. 1293-1298. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Johnson, D.R., G.E. Studebaker, G. Lorenz, and J.K. Greene. 2002. Cotton insect management. University of Arkansas Cooperative Extension Service. http://www.uaex. Edu/Others_Areas/publications/PDF/FSA-2065.PDF.

Jones, J.E. 1963. The growth and fruiting habits of the cotton plant, pp. 3-7. *In* J.S. Roussel and B.J. Baker [eds.], Louisiana cotton production handbook. Louisiana Agric. Expt. Sta., Baton Rouge, LA.

Jones, W.A. and M.J. Sullivan. **1981.** Overwintering habitats, spring emergence patterns, and winter mortality of some South Carolina Hemiptera. Environ. Entomol. 10: 409-414.

1982. Role of host plants in population dynamics of stink bug pests of soybean in South Carolina. Environ. Entomol. 10: 409-414.

Kanga, L.H.B, F.W. Plapp, M.L. Wall, M.A. Karner, R.L. Huffman, T.W. Fuchs, G.W. Elzen, and J.L. Martinez-Carrillo. 1995. Monitoring tolerance to insecticides in boll weevil populations (Coleoptera: Curculionidae) from Texas, Arkansas, Oklahoma, Mississippi, and Mexico. J. Econ. Entomol. 88: 198-204.

Kirkpatrick, T.L. and C.S. Rothrock. 2001. Compendium of cotton diseases. Second Edition. American Phytopathological Society, St. Paul, MN. 74 pp.

Layton, M.B. 2002. Cotton insect control guide, 2002. Mississippi State University Extension Service. Pub No. 343. http://msucares.com/pubs/pub343.htm

Lee, G.R., P. Roberts, and M.A. Abney. **1999.** Stink bugs in cotton: feeding and injury observations, pp. 1036-1038. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B.R. and K. Emfinger. 2002. Insects in low spray environments and modified cotton ecosystems, CD-ROM A010.pdf. *In* P. Dugger and D. Richter [eds.], Proc. 2002 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B.R., J.B. Graves, and P.C. Ellsworth. 1999. Insect and mite pests of cotton. *In* C.W. Smith and [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, New York, NY.

Lockwood, J.A. and R.N. Story. 1986. Adaptive functions of nymphal aggregation in the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Environ. Entomol. 15: 739-749.

Mascarenhas, V.J., F. Shotkoski, and R. Boykin. 2003. Field performance of VIP cotton against various lepidopteran pests in the U.S, pp. 1316-1322. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

McPherson, J.E. and R.M. McPherson. 2000. Stink bugs of economic importance in America north of Mexico. CRC Press, Boca Raton, FL. 253 pp.

McPherson, R.M., J.B. Graves, and T.A. Allain. 1979a. Dosage-mortality responses and field control of seven pentatomids associated with soybean exposed to methyl parathion. Environ. Entomol. 8: 1041-1043.

McPherson, R.M., L.D. Newsom, and B.F. Farthing. 1979b. Evaluation of four stink bug species from three genera affecting yield and quality in Louisiana. J. Econ. Entomol. 72: 188-194.

McPherson, R.M., J.W. Todd, and K.V. Yeragan. 1994. Stink bugs, pp. 87-90. *In* L.G. Higley and D.J. Boethel [eds.], Handbook of soybean insect pests. Entomological Society of America, Lanham, MD.

Mink, J.S., D.J. Boethel, and B.R. Leonard. 1993. Monitoring permethrin resistance in soybean looper (Lepidoptera: Noctuidae) adults. J. Entomol. Sci. 28: 43-50.

Munyaneza, J. and J.E. McPherson. 1994. Comparative study of life histories, laboratory rearing, and immature stages of *Euschistus servus* and *Euschistus variolarius* (Hemiptera: Pentatomidae). Great Lakes Entomol. 26: 263-274.

Negron, J.F. and T.J. Riley. 1987. Southern green stink bug, *Nezara viridula* (Heteroptera: Pentatomidae), feeding in corn. J. Econ. Entomol. 80: 666-669.

Oosterhuis, D.M. and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant, pp. 175-206. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Osteen, C.D. and M. Padgitt. 2002. Economic issues of agricultural pesticide use and policy in the United States, pp. 59-95. *In* W.B. Wheeler [ed.], Pesticides in agriculture and the environment. Marcel Dekker, Inc., New York, NY. 330 pp.

Panizzi, A.R. 1997. Wild hosts of pentatomids: ecological status and role in their status on crops. Annu. Rev. Entomol. 42: 99-122.

Panizzi, A.R. and A.M. Meneguim. 1989. Performance of nymphal and adult *Nezara viridula* on selected alternate host plants. Entomol. Exp. Appl. 50: 215-223.

Parker, E.A. 1941. The biology of the brown cotton bug, *Euschistus servus* (Say). M.S. thesis, Louisiana State University, Baton Rouge, LA. 31 pp.

Patrick, C.R. and G.L. Lentz. 2001. 2001 Cotton insect control guide. University of Tennessee Agricultural Extension Service. Pub No. PB387-1M-1/01.

Pedigo, L.P. and L.G. Higley. 1992. The economic injury level concept and environmental quality: a new perspective. Amer. Entomol. 38: 12-33.

Plapp, F.W., G.M. McWhorter, and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas–1986, pp. 324-326. *In* T. Nelson [ed.], Proc. 1987 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roach, S.C. 1988. Stink bugs in cotton and estimation of damage caused by their feeding on fruiting structures, pp. 292-293. *In* J. Brown and D. Richter [eds.], Proc. 1988 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roberts, P. 1999. Observations of emerging pests in low spray environments, pp. 1034. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roberts, P.M., D. Jones, and J. Ruberson. 2003. 2003 Georgia pest management handbook. http://www.ent.uga.edu/pmh/Agronomic/Agronomic_Crops.htm.

Rolston, L.H. and R.L. Kendrick. 1961. Biology of the brown stink bug, *Euschistus servus* (Say). J. Kansas. Entomol. Soc. 34: 151-157.

Roof, M.E. and F. Arnette. **2000.** Monitoring stink bugs in cotton in South Carolina, pp. 1122-1123. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Russell, J.S. 1999. Effects of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feeding on cotton boll abscission and yield. M.S. thesis, Louisiana State University, Baton Rouge, LA. 47 pp.

Russin, J.S., M.B. Layton, D.B. Orr, and D.J. Boethel. 1988. Stink bug damage to soybean. La. Agric. 32: 3, 24.

Smith, C.W. 1995. Cotton, *Gossypium hirsutum* L., pp. 287-349. *In* C.W. Smith [ed.], Crop production: evolution, history, and technology. John Wiley and Sons, New York, NY.

Smith, M.S. 1990. Influence of insect damage and environmental stresses on aflatoxin production in Louisiana preharvest field corn. M.S. thesis, Louisiana State University, Baton Rouge, LA. 93 pp.

Smith, R.F., J.L. Apple, and D.G. Bottrell. 1976. The origins of integrated pest management concepts for agricultural crops. *In* J.L. Apple and R.F. Smith [eds.], Integrated pest management. Plenum Press, New York, NY.

Snodgrass, G.L. 1996. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). J. Econ. Entomol. 89: 1053-1059.

Sprenkel, R.K. 2002. Insect management in cotton. University of Florida Cooperative Extension Service, Pub. No. ENY400. http://edis.ifas.ufl.edu/BODY_IG059

Stewart, S.D., J.J. Adamczyk, K.S. Knighten, and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis*: baseline on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Thompson, G.D., P.W. Borth, M.C. Shaw, R.W. Huckaba, B.A., L.G. Peterson, J.M. Richardson, and D.J. Porteons. 1996. Spinosad and the new naturalyte insect control class, pp. 870-872. *In* D. Richter and J. Armour [eds.], Proc. 1996 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Todd, J.W. 1976. Effects of stink bug feeding on soybean seed quality, pp. 611-618. World Soybean Research Conference.

Todd, J.W. 1989. Ecology and behavior of *Nezara viridula*. Annu. Rev. Entomol. 34: 273-292.

Todd, J.W. and D.C. Herzog. 1980. Sampling phytophagous pentatomidae on soybean. *In* M. Kogan and D.C. Herzog [eds.], Sampling methods in soybean entomology. Springer-Verlag, New York, NY.

Toscano, N.C. and V.N. Stern. 1976. Cotton yield and quality loss caused by various levels of stink bug infestations. J. Econ. Entomol. 69: 53-56.

Townsend, L.H. and J.D. Sedlacek. 1986. Damage to corn caused by *Euschistus servus*, *Euschistus variolarius*, and *Acrosternum hilare* (Heteroptera: Pentatomidae) under greenhouse conditions. J. Econ. Entomol. 79: 1254-1258.

Tugwell, P., S.C. Young, B.A. Dumas, and J.R. Phillips. 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury, and significance of wild hosts. Arkansas Expt. Sta. Report, Series 227. 24 pp.

Turnipseed, S.G., M.J. Sullivan, J.E. Mann, and M.E. Roof. 1995. Secondary pests in transgenic Bt cotton in South Carolina, pp. 768-769. *In* D. Richter and J. Armour [eds.], Proc. 1995 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Turnipseed, S.G., M.J. Sullivan, A. Hagerty, and R. Ridge. 2001. Cotton as a model IPM system in the southeast: a dream or potential reality, pp. 1009-1010. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

United States Department of Agriculture [USDA], Farm Service Agency. 2001. The conservation reserve program. http://www.fsa.usda.gov/crpstorpt107/la.htm.

Velasco, L.R.I. and G.H. Walter. 1992. Availability of different host plant species and changing abundance of the polyphagous bug *Nezara viridula* (Hemiptera: Pentatomidae). J. Econ. Entomol. 21: 751-759.

Wene, G.P. and L.W. Sheets. 1964. Notes on control of stink bugs affecting cotton in Arizona. J. Econ. Entomol. 57: 60-62.

Williams, M.R. 1994. Cotton insect loss estimates, 1993, pp. 743-763. *In* D. Richter and J. Armour [eds.], Proc. 1994 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M.R. 1996. Cotton insect loss estimates, 1995, pp. 670-689. *In* D. Richter and J. Armour [eds.], Proc. 1996 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M.R. 2001. Cotton insect loss estimates, 2000, pp. 774-776. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M.R. 2003. Cotton insect loss estimates, 2002, pp. 1217-1273, CD-ROM H059a.pdf. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Wing, K.D., M. Sacher, Y. Kagaya, Y. Tsurubuchi, M. Connair, and M. Schnee. 2000. Bioactivation and mode of action of oxadiazine indoxacarb in insects. Crop Protection 19: 537-545.

Willrich, M.M., C.G. Clemens, M.E. Baur, B.J. Fitzpatrick, and D.J. Boethel. 2000. Evaluation of insecticides for southern green and brown stink bug control on soybean, 1999. Arthropod Management Tests 25: 314.

Yeargan, K.V. 1977. Effects of green stink bug damage on yield and quality of soybeans. J. Econ. Entomol. 70: 619-622.

CHAPTER 2

INJURY TO PRE-FLOWERING AND FLOWERING COTTON BY BROWN STINK BUG AND SOUTHERN GREEN STINK BUG*

Introduction

Hemipteran pests of cotton, *Gossypium hirsutum* L., have become significant pests in the mid-southern and southeastern United States. Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say), are the most common species in this complex. *Lygus* spp. infested 8,111,090 and stink bugs infested 6,180,966 acres across the seventeen states of the United States cotton belt in 2001, ranking third and fourth, respectively, among all insect pests (Williams 2002). The increased occurrence of hemipterans is related to the reduction of broad-spectrum insecticide applications against key cotton pests, the use of target-selective insecticides, adoption of Bollgard cotton, and producer participation in boll weevil, *Anthonomous grandis grandis* Boheman, eradication programs (Greene and Herzog 1999, Leonard et al. 1999, Roberts 1999). In the past, insecticides that targeted boll weevil and other key cotton pests coincidently controlled infestations of tarnished plant bugs and stink bugs (Layton 2000).

Cotton seedlings and flower buds (squares) are particularly sensitive to injury from tarnished plant bug (Tugwell et al. 1976). Tarnished plant bug feeding in the terminal of cotton seedlings can cause the terminal to abort (Tugwell et al. 1976). Small (pin-head and match-head) squares and bolls fed on by tarnished plant bug can abscise from the plant (Tugwell et al. 1976, Russell 1999). Larger squares will not abscise from the plant upon feeding; however, the ensuing flower may be malformed (necrotic anthers and corolla) (Tugwell et al. 1976, Burris et

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al. 1997). Generally, in the mid-southern and southeastern United States, tarnished plant bug infestations are controlled in pre-flowering cotton when first position square retention on sympodial branches falls below an acceptable level (70-80%) and adults or nymphs are present.

Most of the data collected on stink bug (particularly southern green stink bug and green stink bug) injury to cotton is related to their occurrence during boll development stages. Stink bugs can cause small bolls to abscise, decrease seedcotton yields, reduce lint quality, and inhibit seed germination (Wene and Sheets 1964b, Barbour et al. 1990, Greene et al. 1999). Limited data has been published on the effects of stink bugs on cotton seedlings, or on squares. However, the recent changes in Integrated Pest Management practices have increased the occurrence of stink bugs in cotton fields from seedling emergence until harvest.

Presently, concerns exist about the effects of stink bugs on pre-flowering cotton plants and squares, and at what age a boll is tolerant to injury. Therefore, studies were conducted to determine the effects of brown stink bug and southern green stink bug on cotton plant seedlings and reproductive structures of various ages.

Materials and Methods

Study Site and Plant Material

These studies were conducted at the Macon Ridge Research Station near Winnsboro, Louisiana (Franklin Parish) during 2001, 2002, and 2003. The soil was a Gigger-Gilbert silt loam complex. Infestation studies on cotton seedlings (pre-squaring), cotton with small (match-head) squares, and individual fruiting structures (squares and bolls) were planted to 'DP458BR' in 2001 and 2003, and 'Stoneville 4892BR' in 2002. Cultural practices and integrated pest management strategies recommended by the LSU AgCenter were used to maintain the plots for optimum productivity. No supplemental irrigation was applied to plots.

Insects

Brown stink bug and southern green stink bug adults and nymphs were collected early-season (May and June) from mustard, *Brassica* spp., and field corn, *Zea mays* L. Late-season (July and August) colonies were established from soybean, *Glycine max* (L.) Merrill. Insects were collected using a standard 38.1 cm diameter sweep net or removed from plants by hand. Insects were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan) for 24 h and fed washed green beans, *Phaseolus vulgaris* (L.), and peanut, *Arachis hypogae* L., seeds. After the 24 h period, a cohort of stink bugs were selected that displayed normal behavior, without obvious signs of physical injury or parasitism (Todd 1989). Individual stink bugs were placed in nylon no. 280 mesh cages (bags) and transported to the field in a chilled cooler to eliminate mortality from heat stress. In the field, cages were used to contain individual stink bugs on plant structures. The size of the cage varied depending on the type of plant structure infested.

Infestation of Adults on Pre-flowering Cotton Plants

The two treatments in these studies included stink bug-infested and non-infested plants. For infestation studies on cotton seedlings (plant terminals including two to three main stem nodes above the cotyledon), match-head squares (first position on a sympodial branch, seven to eight main stem nodes above the cotyledon, 3 to 4 mm in diameter), and large squares (>8 mm diameter, 15 to 16 d old squares on the first position of a sympodial branch, nine to 15 main stem nodes above the cotyledon), the experimental design was a randomized block, with infestation dates as blocks. Similar, adjacent plants within each block (date) were paired and were randomly infested or non-infested. Non-infested plants also had cages placed over each respective structure. Data for all variables were analyzed using paired t-test procedures by

comparing infested and non-infested plants ($\alpha = 0.05$) (PROC TTEST, SAS Institute 1998). Data for 2001, 2002, and 2003 were pooled for analysis. Experiments on pre-flowering cotton for brown stink bug adults were duplicated for southern green stink bug adults. Sample sizes ranged from 43 to 154 paired plants for each stink bug species.

One adult per cage [15 cm x 15 cm (length x width)] was confined to the plant terminal on a cotton seedling. A plant terminal was defined as the uppermost, two to three main stem nodes of the plant containing the apical meristem. The opening of the cage was tightly closed around the main stem of the plant with a drawstring. Stink bugs were caged on each plant for 7 d after which time the cages and insects were removed. At 7, 14, and 21 d after infestation (DAI), plants were observed for aborted terminals and main stem height (cm) was recorded. Height on an individual plant was measured from the soil surface to the tip of the terminal. At 21 DAI, square retention (total number of squares/total number of fruiting sites) and total number of main stem nodes per plant were recorded. Individual plant heights and number of nodes were used to calculate height to node ratios (HNR) [plant height (cm) / number of nodes)] and provide an estimate of sympodial development.

On plants with a small (match-head) square, infestations were performed similar to cotton seedling infestations, except cages measured 17.5 cm x 16 cm and the duration of infestation was 5 d. Each caged plant terminal contained one match-head square. Square retention for each plant was measured at 5, 12, and 19 DAI. The number of days from planting to first flower and the growth stage corresponding to that date (total number of main stem nodes above the cotyledon) was determined.

Individual large squares on cotton plants were infested with one adult per cage (15 cm x 15 cm). The opening of the cage was tightly closed around the peduncle of the square with a

drawstring. The duration of each infestation was 5 d. At 9 d after mesh cages were removed (14 DAI), infested and non-infested plants were assessed for percent square abscission, flowers with necrotic anthers, and boll abscission.

Infestation of Nymphs on Cotton Squares

The two treatments in these studies were stink bug-infested and non-infested plants. The experimental design and data analysis for these studies was similar to that described for adult infestations. Multiple squares on cotton plants (growth stage of five to seven main stem nodes above a flower located on the first position of a sympodial branch) were infested with two stages of southern green stink bug nymphs. A medium square (ca. 6 mm diameter, 12 to 13 d old on the first position of a sympodial branch) and a small square (ca. 4 mm diameter, on the second position on the same sympodial branch as the medium square) were infested. Cages (15 cm x 15 cm) containing either third instar (two/cage) or fourth-fifth instar (one/cage) stink bugs were placed on a fruiting branch containing both squares. The opening of the cage was tightly closed around the sympodial branch between the main stem and the first position on the sympodial branch. The duration of each infestation was 7 d. Abscission was recorded for medium and small squares. Medium squares were further monitored for occurrence of a flower with necrotic anthers, occurrence of a boll, and boll abscission. A total of 84 and 105 paired plants were used for third instar and fourth-fifth instar infestations, respectively.

Brown Stink Bug Adults Infested on Bolls

Cotton plants were monitored bi-weekly until the first week of flowering. First position flowers (flower located on the first position of a sympodial branch from the main stem of the plant) were marked with a yellow “snap-on-tag” (A.M. Leonard, Inc. Piqua, Ohio) placed on the sympodial branch between the peduncle of the flower and the main stem of the plant. The date

of anthesis was recorded on the tag in permanent ink to ascertain boll age at the time of infestation. Boll age was calculated using heat unit accumulation beginning at anthesis, as described by Bagwell and Tugwell (1992). Heat units were calculated for each day of infestation as: [(maximum daily temperature + minimum daily temperature)/2]-15.5, where 15.5°C (60°F) is the minimum adequate temperature for cotton plant development.

The two treatments in these studies were stink bug-infested and non-infested flowers or bolls. The treatments were arranged in a completely randomized design. Infestation procedures were similar to that used by Adamczyk et al. (1998) and Russell (1999) for caging lepidopteran larvae and tarnished plant bug, respectively, on cotton bolls. One brown stink bug adult per cage was placed on an individual boll using the same procedures for large square infestations. For each infested boll, a blue "snap-on-tag" was also placed in the same position as the yellow "snap-on-tag" and labeled with the date of infestation and the date of white flower. Non-infested treatments consisted of cages placed on bolls, with blue "snap-on-tags" placed on the sympodial branch labeled as the control with the corresponding date of infestation and date of flower. On each infestation date, equal numbers of infested and non-infested bolls were used. Stink bugs were caged on each boll for 72 h, at which time the cages and insects were removed. Stink bug infestations were initiated at flower (0 heat units) and continued through 892 heat units beyond anthesis.

In 2002, the diameter of each non-abscised boll was recorded using a dial caliper (Forestry Suppliers, Inc., Jackson, MS) at 72 h after infestation (HAI). Individual boll measurements were taken at the widest diameter using two diametrically opposite points. Boll diameter was also measured on bolls without cages (controls) using the same procedure as described above; however, bolls were not paired with infested and non-infested bolls. A total of

90 bolls were measured for ca. 25 d, each at intervals of two to three days.

The number of abscised bolls was recorded at 72 HAI and at harvest. All harvestable bolls were individually collected and seedcotton weights were recorded. In 2002, the proportion of hard locked carpels [carpel (locule) with lint visible, but not fluffed open sufficiently to be harvested with a mechanical picker] within each boll was recorded. Cumulative abscission data, individual boll weights, and proportion of hard locked carpels per boll within the same heat unit were grouped into 17 classes of 50 heat units (0-50, 51-100, 101-150, through 851-900). Seedcotton from bolls infested at the same age and on the same date were separated into lint and seed with a laboratory gin. Seed were grouped into nine classes of 100 heat units (0-100, 101-200, 201-300, through 801-900) to ensure an adequate sample size for germination tests. The standard warm germination test for cotton seed was used, which measures the percentage of seedlings that have a combined hypocotyl and root length of 3.75 cm (Association of Official Seed Analysts 2000).

For each heat unit class, boll size, seedcotton yield, hard locked carpels, and seed germination were analyzed using a paired t-test by comparing diameter, weight, proportion, and percent germination respectively, of infested bolls to those of non-infested bolls (PROC TTEST, SAS Institute 1998). Boll diameter data was also analyzed using regression analysis (PROC REG, SAS Institute 1998). Within infested, non-infested, and non-caged treatments, diameter (dependent variable) was plotted against heat unit accumulated (independent variable) of that same boll on the day cages were removed. Boll abscission data for infested bolls was corrected for natural abscission in the non-infested bolls using Abbott's formula (Abbott 1925) and analyzed using regression analysis (PROC REG, SAS Institute 1998). Corrected abscission (dependent variable) data was plotted against accumulated heat units (independent variable) of

infested bolls to determine regression equations describing the relationship. The analytical model included only those heat units in which abscission occurred. The data for all variables measured in 2001 and 2002 were pooled for analysis. A total of 480 and 555 plants pairs were used in 2001 and 2002, respectively.

Results and Discussion

Infestation of Adults on Pre-flowering Cotton Plants

There were no significant differences in plant height on 7, 14, and 21 DAI between cotton seedlings (pre-squaring) that were infested with brown stink bug or southern green stink bug adults and the non-infested plants ($P > 0.05$) (Table 2.1, 2.2). Additionally, there were no differences in HNR and square retention between infested and non-infested plants at 21 DAI ($P > 0.05$) (Table 2.1, 2.2). No aborted terminals or atypical main stem development were observed in the infested or non-infested treatment.

Square retention on cotton with match-head squares on 5, 12, and 19 DAI was not significantly different between brown stink bug or southern green stink bug-infested and non-infested plants ($P > 0.05$) (Table 2.1, 2.2). The number of days after planting to a first flower was not significantly different between infested and non-infested plants ($P > 0.05$) (Table 2.1, 2.2). Additionally, the plant growth stage (number of main stem nodes above the cotyledon) during which that flower appeared was not significantly different between treatments ($P > 0.05$) (Table 2.1, 2.2).

Results from studies with tarnished plant bug were different from that observed for brown stink bug and southern green stink bug in these studies. Significant reductions in plant height were observed after 72 h infestations of tarnished plant bug (1 adult per plant) on cotyledons and cotton with 2, 4, and 6 nodes above the cotyledon (Hanny et al. 1977).

Table 2.1. Response of pre-flowering cotton plants and flower buds to infestations of brown stink bug adults.

			Mean ± SD				
Stage[1]	Variable	DAI[2]	Infested	Non-infested	df	*t*	*P* > *t*
Seedlings	Height (cm)	7	23.4 ± 4.6	23.5 ± 5.3	146	-0.09	0.93
		14	32.4 ± 5.8	31.6 ± 6.6	146	0.74	0.46
		21	46.1 ± 4.8	45.0 ± 6.4	146	1.13	0.26
	Height:Node	21	4.9 ± 0.6	4.8 ± 0.8	146	0.4	0.69
	Square retention (%)	21	73.8 ± 24.9	68.2 ± 29.0	146	1.26	0.21
Match-head square	Square retention (%)	5	88.6 ± 15.7	90.7 ± 18.5	138	-0.73	0.47
		12	69.7 ± 17.2	71.8 ± 13.7	138	-0.78	0.44
		19	62.4 ± 16.3	65.3 ± 13.9	138	-1.13	0.26
	Flower Initiation[3]						
	Node	-----	11.1 ± 1.2	11.1 ± 1.1	138	0.07	0.94
	DAP	-----	63.6 ± 3.7	64.0 ± 3.3	138	-0.68	0.50

Means within rows are compared using paired t-tests ($\alpha = 0.05$).
[1]Seedling infestations: n = 74 pairs; match-head square infestation: n = 70 pairs.
[2]Days after infestation.
[3]Main stem nodes above the cotyledon (Node) and days after planting (DAP).

Table 2.2. Response of pre-flowering cotton plants and flower buds to infestations of southern green stink bug adults.

			Mean ± SD				
Stage[1]	Variable	DAI[2]	Infested	Non-infested	df	*t*	*P* > *t*
Seedlings	Height (cm)	7	24.1 ± 3.0	24.6 ± 3.3	170	1.28	0.20
		14	29.5 ± 4.8	29.7 ± 4.8	170	0.42	0.68
		21	32.8 ± 6.1	32.8 ± 5.6	170	0.02	0.98
	Height:Node	21	3.5 ± 0.5	3.5 ± 0.6	170	0.10	0.92
	Square retention (%)	21	49.1 ± 24.5	45.9 ± 23.3	170	-0.90	0.37
Match-head square	Square retention (%)	5	87.6 ± 21.4	91.3 ± 17.3	84	0.87	0.38
		12	78.9 ± 13.3	73.6 ± 15.7	84	-1.69	0.10
		19	76.1 ± 10.7	76.2 ± 10.4	84	0.04	0.97

Teble 2.2. Continued.

Flower Initiation[3]						
Node	-----	14.7 ± 1.2	14.9 ± 1.2	84	1.00	0.32
DAP	-----	70.4 ± 2.3	70.4 ± 2.4	84	0.03	0.98

Means within rows are compared using paired t-tests ($\alpha = 0.05$).
[1]Seedling infestations: n = 86 pairs; match-head square infestation: n = 43 pairs.
[2]Days after infestation.
[3]Main stem nodes above the cotyledon (Node) and days after planting (DAP).

Infestations of three bugs per plant for 22 d on seedling cotton (one fully expanded leaf) reduced plant height through 9 weeks after infestation. Tarnished plant bug (one to three adults per plant) feeding has caused the abortion of terminals in 87 to 98% of infested plants (Wene and Sheets 1964a, Hanny et al. 1977). Injury by tarnished plant bugs during pre-squaring growth stages also delayed the initiation of sympodial branches (Hanny et al. 1977).

Square abscission between plants infested with brown stink bug or southern green stink bug adults and non-infested plants was not significantly different ($P > 0.05$) (Table 2.3). Squares that did not abscise within each treatment became a white flower. On brown stink bug and southern green stink bug infested plants, 29.0 and 33.3% respectively, of the white flowers were produced from squares on 2, 3, and 4 DAI. Flowers produced on these days were bolls on the day that cages were removed because anthesis occurs within a 24 h period. White flowers were examined for necrotic anthers and no significant differences ($P > 0.05$) were observed between infested and non-infested plants (Table 2.3). Fruiting forms were examined daily until 14 DAI and there was no significant difference in boll abscission between infested and non-infested plants ($P > 0.05$) (Table 2.3). Although boll abscission was not significantly different between infested and non-infested plants, the larger proportion of boll abscission in infested plants was due to the larger proportion of bolls present on the day cages were removed (Table 2.3). Plant development during the study allowed stink bugs to be offered bolls that were produced from

squares that were initially infested. Therefore, stink bug feeding induced boll abscission.

Table 2.3. Response of large squares (pre-candle, >8 mm diameter) to infestations of brown stink bug and southern green stink bug adults.

	Brown Stink Bug (Mean ± SD)[1]					Southern Green Stink Bug (Mean ± SD)[2]				
Variable	Infested	Non-infested	df	*t*	*P* > *t*	Infested	Non-infested	df	*t*	*P* > *t*
Square abscission (%)	3.0 ± 1.3	1.5 ± 0.8	18	0.97	0.35	5.0 ± 5.3	0 ± 0	6	1.0	0.36
Presence of necrotic anthers (%)	4.2 ± 1.9	0.5 ± 1.7	18	1.86	0.09	4.7 ± 9.4	6.6 ± 4.7	6	-0.36	0.73
Boll abscission (%)	13.6 ± 3.9	6.4 ± 3.3	18	1.4	0.18	29.1 ± 19.9	13.8 ± 11.1	6	1.35	0.23

Means within species and rows are compared using a paired t-test ($\alpha = 0.05$).
[1]n = 154 pairs.
[2]n = 51 pairs.

Pack and Tugwell (1976) demonstrated tarnished plant bug and clouded plant bug, *Neurocolpus nubilus* (Say), (one adult or nymph/plant) induced 18% abscission of squares 3 mm or larger after 24 h of exposure. Tarnished plant bug injury also has induced darkened anthers in non-abscised squares (Pack and Tugwell 1976, Tugwell et al. 1976). Squares exhibiting 60 to 90% of the total anthers damaged resulted in 67% boll abscission (Pack and Tugwell 1976). Inadequate pollination was believed to cause subsequent boll abscission (Pack and Tugwell 1976). In our studies, brown stink bug and southern green stink bug were initially infested on squares, but later were exposed for a short duration to flowers and small bolls, resulting in no abscission and abscission, respectively. In cotton, flowers typically do not abscise, but bolls ca. five to ten d beyond anthesis are very sensitive to abscission (Guinn 1986). Bolls that mature to 18 d beyond anthesis demonstrate low rates of abscission (Guinn 1986).

Infestation of Nymphs on Cotton Squares

For third instar southern green stink bug, there was no significant difference among treatments in abscission of first position, medium size squares or second position, small squares (Table 2.4). Abscission of medium and small squares by fourth-fifth instar southern green stink

Table 2.4. Abscission (%) of medium and small squares as influenced by infestations of southern green stink bug nymphs.

	Third Instar (Mean ± SD)					Fourth-fifth Instar (Mean ± SD)				
Square Size	Infested	Non-infested	df	*t*	*P* > *t*	Infested	Non-infested	df	*t*	*P* > *t*
Medium[1]	23.1 ± 11.1	0.0 ± 0.0	4	2.08	0.0532	17.6 ± 8.9	5.9 ± 3.4	4	1.22	0.2889
Small squares[2]	28.4 ± 12.1	15.5 ± 10.9	4	0.80	0.4699	31.4 ± 7.1	27.4 ± 2.0	4	0.53	0.6213

Means within life stages and rows are compared using a paired t-test ($\alpha = 0.05$).
[1]Square on the first node of a fruiting branch (ca. 6 mm diameter).
[2]Square on the second node of a fruiting branch (ca. 4 mm diameter).

bug was not significantly different. However, for both stages of nymphs, abscission of medium squares (ca. 6 mm diameter) in the infested treatment was consistently higher. The results of third instar infestations on first position squares of medium size for ≥ 7 d suggest nymphs (≥1 nymph/fruiting branch) may induce abscission in some instances. However, in all other infestation studies that occurred on squares of any size (brown stink bug and southern green stink bug adults on match-head and large squares, and nymphs on squares), square abscission was not significantly different ($P \geq 0.1$). Additionally, a large proportion of second position (ca. 4 mm diameter) small squares abscised from the plant, regardless of treatment. In cotton, abscission of squares and young bolls can be a natural occurrence and is accentuated by overcast weather, extreme temperatures, and water stress (Oosterhuis and Jernstedt 1999). Also, abscission rates of squares and bolls on second positions of a sympodial branches are higher because first position fruiting is more competitive for assimilates during the stage of development when they are most vulnerable to abscise (Cothren 1999). In these studies, abscission data for second position structures on infested and non-infested fruiting branches confirm these findings.

Non-abscised squares in infested and non-infested treatments became a flower. Flowers

were examined for necrotic anthers and no significant differences between infested and non-infested plants were observed [(Infested third instar: 17.2 ± 11.8, Non-infested: 3.0 ± 5.3, df = 4, t = 1.91, P = 0.0647); (Infested fourth-fifth instar: 13.7 ± 14.8, Non-infested: 3.9 ± 3.4, df = 4, t = 1.12, P = 0.1631)]. White flowers in all treatments pollinated normally and eventually produced bolls. There were no significant differences in boll abscission between infested and non-infested plants [(Infested third instar: 18.6 ± 9.5, Non-infested: 9.2 ± 0.8, df = 4, t = 1.71, P = 0.081); (Infested fourth-fifth instar: 13.7 ± 14.8, Non-infested: 7.9 ± 3.4, df = 4, t = 0.67, P = 0.2700)].

Brown Stink Bug Adults Infested on Bolls

There was a negative linear relationship describing cumulative abscission of infested bolls (F = 7.09; df = 1,5, P < 0.0448) as a function of heat unit accumulation (Figure 2.1). Boll abscission ranged from 50.9% for bolls infested at 51 to 100 heat units to 0% for bolls infested at ≥ 351 heat units (14.0 d; based upon each day accumulating ca. 25 heat units) beyond the date of anthesis. Abscission of small bolls from feeding by the Say stink bug, *Chlorochroa sayi* Stål, *Euschistus impectiventris* Stål, green stink bug, and brown stink bug has been documented by several researchers (Wene and Sheets 1964b, Barbour et al. 1990, Fromme 2000). In other studies with brown stink bug, all infested bolls 3 d beyond anthesis abscised (Fromme 2000). Abscission of young bolls is also characteristic of injury by tarnished plant bug (Burris et al. 1997, Russell 1999).

Seedcotton yields for bolls infested with brown stink bug were significantly reduced (P < 0.05) compared to non-infested bolls for age classes infested from 0 to 550 heat units (22.0 d) beyond anthesis (Figure 2.2). Mean weight per boll through 550 heat units beyond anthesis in the infested and non-infested treatment was 3.247 and 4.265 grams, respectively. No significant

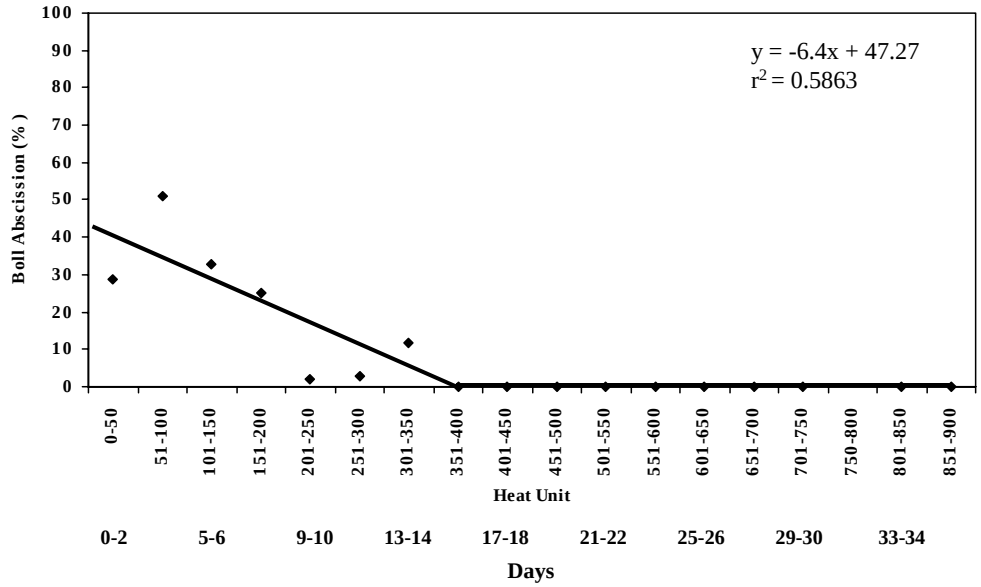

Figure 2.1. Relationship between boll abscission induced by brown stink bug and accumulated heat units [or days (based on the accumulation of ca. 25 heat units per day)] after anthesis, 2001 and 2002.

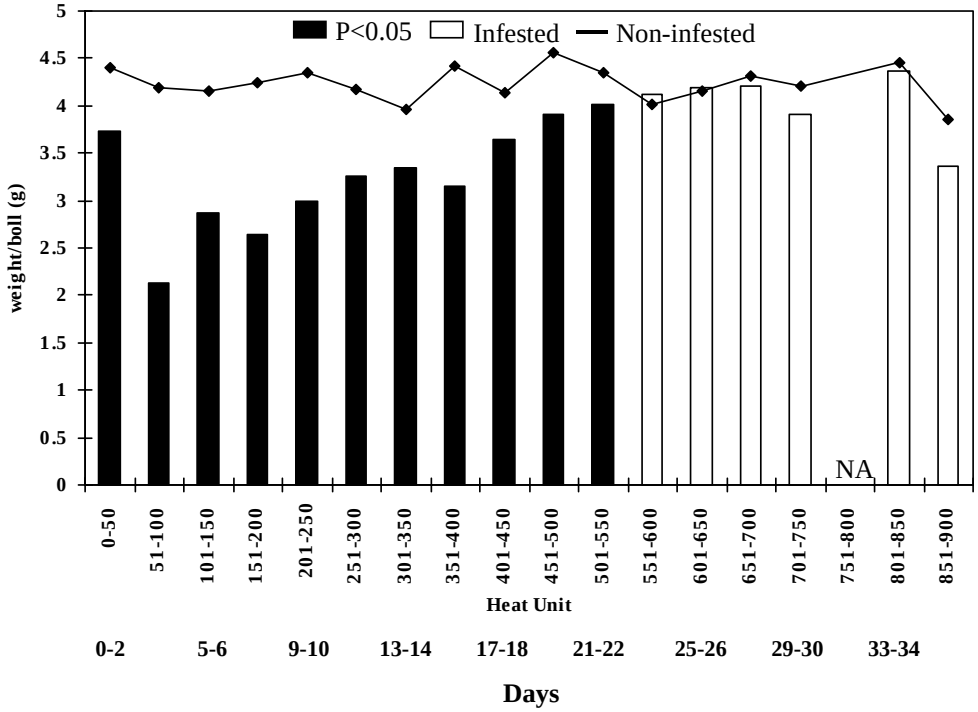

Figure 2.2. Relationship of heat unit accumulation [or days (based on the accumulation of ca. 25 heat units per day)] and brown stink bug injury on seedcotton yield of individual bolls (bars represent mean weights of infested bolls and the line represents mean weights of non-infested bolls; dark bars represent weights of infested bolls significantly [α =0.05] different from non-infested boll weights [$P < 0.05$]), 2001 and 2002.

reductions in yield were observed for bolls infested after they accumulated > 551 heat units (22.0 d) beyond anthesis. Mean weight per boll across age classes > 551 heat units beyond anthesis was 4.024 and 4.167 g for the infested and non-infested treatment, respectively. Although no stink bug-induced abscission occurred on bolls that accumulated ≥ 351 heat units (14.0 d) beyond the date of anthesis, seedcotton yield was reduced. Significant yield losses were observed for bolls infested after they had accumulated 351 to 550 heat units ($P < 0.05$).

Fromme (2000) observed reductions in seedcotton yield of 59 and 45% in bolls 11 and 14 d beyond anthesis, respectively, after 4 d of brown stink bug (one adult per boll) infestation. The impact of brown stink bug on yield was not evaluated in bolls beyond 14 d (351 heat units) of anthesis; however, destructive sampling indicated the presence of internal injury in bolls up to 17 d beyond anthesis (338 heat units) (Fromme 2000). In similar studies with southern green stink bug, infestations of fifth instar nymphs for 7 d significantly reduced seedcotton yield in bolls aged 4 (74 heat units), 8 (171 heat units), 10 (220 heat units), 13, 14 (315 heat units), 18 (401 heat units), and 21 (472 heat units) d beyond anthesis (Greene et al. 1999, Greene et al. 2001). No significant reductions in seedcotton yield were observed in bolls aged 25 (559 heat units) and 30 (658 heat units) d beyond anthesis (Greene et al. 2001).

In 2002, 32 ages of bolls, ranging from 0 to 823.5 heat units, were infested with adult brown stink bug for a total of 15 age classes of 50 heat units each. The proportion of hard locked carpels within bolls infested with brown stink bug were significantly greater ($P < 0.05$) compared to non-infested bolls for ages classes infested from 51 to 400 heat units (3.0 through 16.0 d) beyond anthesis (Figure 2.3). Mean proportion of hard locked carpels per boll within these age classes was 0.48 and 0.17 for infested and non-infested bolls, respectively. No

significant increases in hard locked carpels were observed for bolls that had been infested in four of the seven age classes > 401 heat units (17.0 d) beyond anthesis. Green stink bug infestations (three adults per plant) have also been associated with reductions in the amount of harvestable cotton as the duration of infestation and number of punctures per boll increased (Barbour et al. 1990).

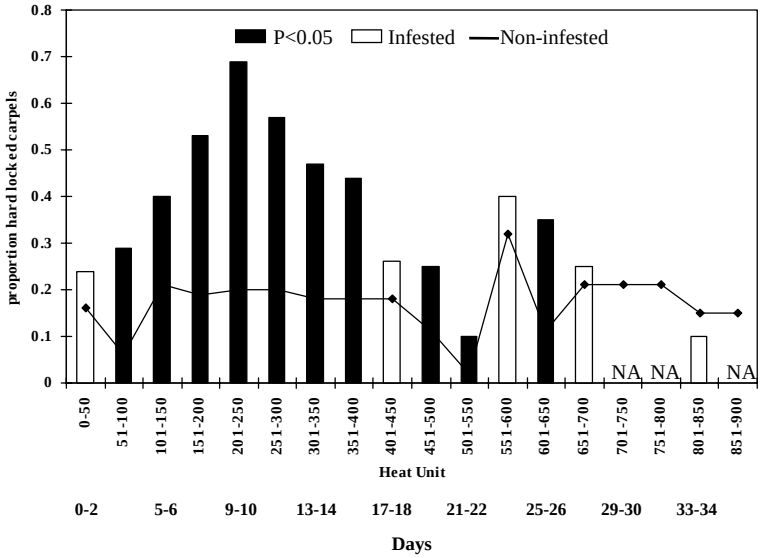

Figure 2.3. Relationship of heat unit accumulation [or days (based on the accumulation of ca. 25 heat units per day)] and brown stink bug injury on proportion of hard locks within individual bolls (bars represent mean proportion of infested bolls and the line represents mean proportion of non-infested bolls; dark bars represent proportions of infested bolls significantly [$\alpha = 0.05$] different from non-infested boll proportions [$P < 0.05$]), 2001 and 2002.

The percentage of seed germinated from bolls previously exposed to brown stink bug was significantly lower ($P < 0.05$) compared to non-infested bolls in age classes infested from 101 to 600 heat units (24.0 d) beyond anthesis (Figure 2.4). Mean germination across this range of ages was 35.2 and 50.8% for infested and non-infested bolls, respectively. No significant reductions in germination were observed for bolls that had been infested in two of the three age classes > 601 heat units (25.0 d) beyond anthesis. Reduced germination has also been observed in whole plant infestations of *E. conspersus* and *Chlorochroa uhleri* Stål, and green stink bug as the

number of stink bugs per plant and duration of exposure increased, respectively (Toscano and Stern 1976, Barbour et al. 1990).

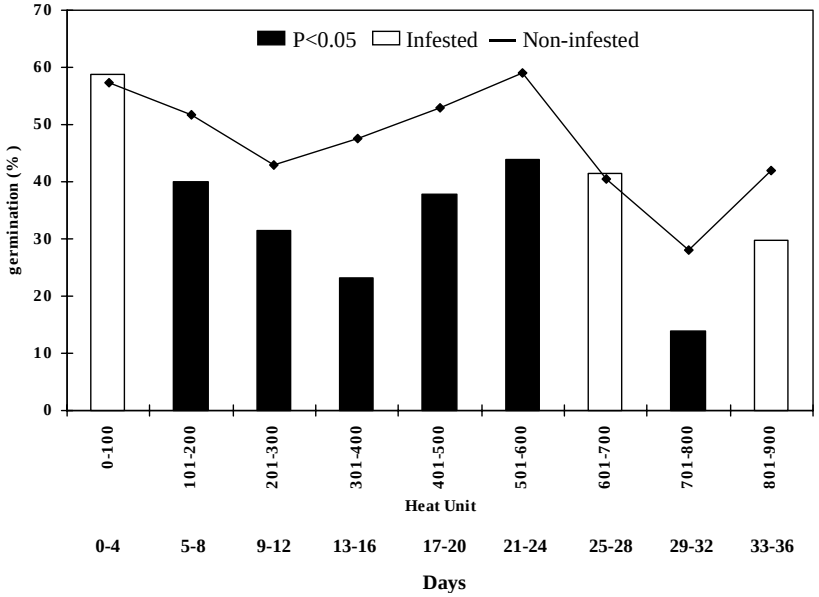

Figure 2.4. Relationship of heat unit accumulation [or days (based on the accumulation of ca. 25 heat units per day)] and brown stink bug injury on seed germination of individual bolls (bars represent mean germination of infested bolls and the line represents mean germination of non-infested bolls; dark bars represent germination of infested bolls significantly [$\alpha = 0.05$] different from non-infested boll germination [$P < 0.05$]), 2001 and 2002.

In 2002, 32 ages of bolls, ranging from 0 to 823.5 heat units, were infested with adult brown stink bug. There was a significant relationship between the age (heat unit) of an individual boll on the day cages were removed and the corresponding diameter (cm) for that same boll when infested [diameter = $0.529 + 9.49\text{x}10^{-3}$heat unit $- 7.5\text{x}10^{-6}$heat unit^2, $P < 0.0001$, r^2=0.87] or non-infested (caged) [diameter = $0.816 + 8.79\text{x}10^{-3}$heat unit $- 7.06\text{x}10^{-6}$heat unit^2, $P < 0.0001$, r^2=0.87] (Figure 2.5). There was also a significant relationship between age and diameter for non-caged bolls [diameter = $0.059 + 1.33\text{x}10^{-2}$heat unit $- 1.34\text{x}10^{-5}$heat unit^2, $P < 0.0001$, $r^2 = 0.94$]. According to values predicted by the model, the maximum diameter and corresponding heat unit for infested, non-infested caged, and non-caged bolls were 3.52 cm

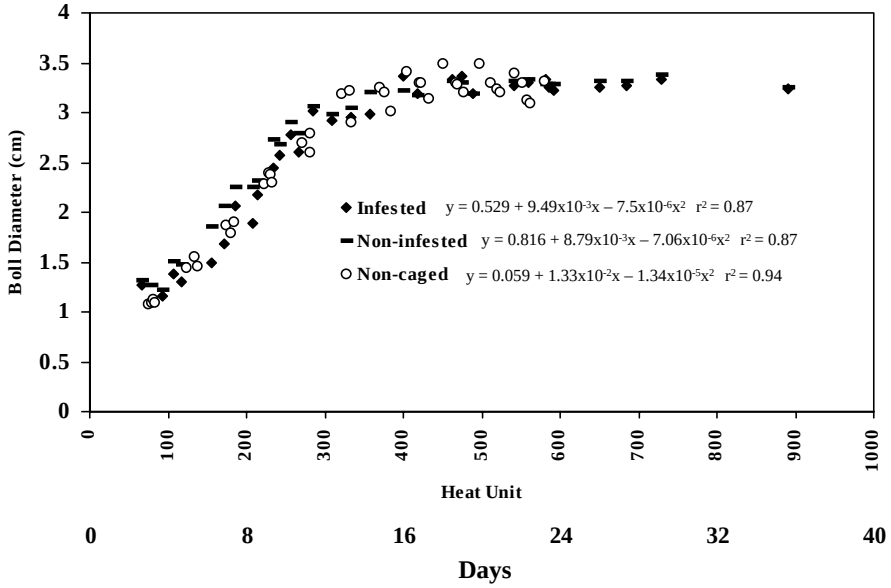

Figure 2.5. Quadratic relationship of heat unit accumulation and boll growth of individual bolls infested with brown stink bug, non-infested, and non-caged ($P < 0.0001$), 2002. Days are based upon the accumulation of ca. 25 heat units per day.

(593.1 heat units), 3.55 cm (622.5 heat units), and 3.33 cm (493.7 heat units), respectively. Non-caged bolls had a lower maximum diameter compared to infested and non-infested caged bolls; however, the relationship describing boll diameter as a function of heat unit accumulation was similar. Measurements on non-caged bolls were temporally separated from infested and non-infested bolls, and may explain the differences in these results. Diameters of bolls infested with brown stink bug were significantly lower compared to non-infested bolls for 12 of the 14 boll age classes infested from 0 to 266.5 heat units (ca. 10 to 11 d) ($P < 0.05$) (Figure 2.6).

Boll size is largely influenced by fiber elongation. Fiber length increases rapidly in the first several days beyond anthesis, with the greatest increase in elongation at 12 d. Final length is attained at 27 d beyond anthesis (Schubert et al. 1973). Leffler (1976) has shown final boll size, as measured by fresh and dry weight, occurs at 21 to 28 d beyond anthesis. In these studies, brown stink bug significantly reduced boll diameter during the period when rate of fiber

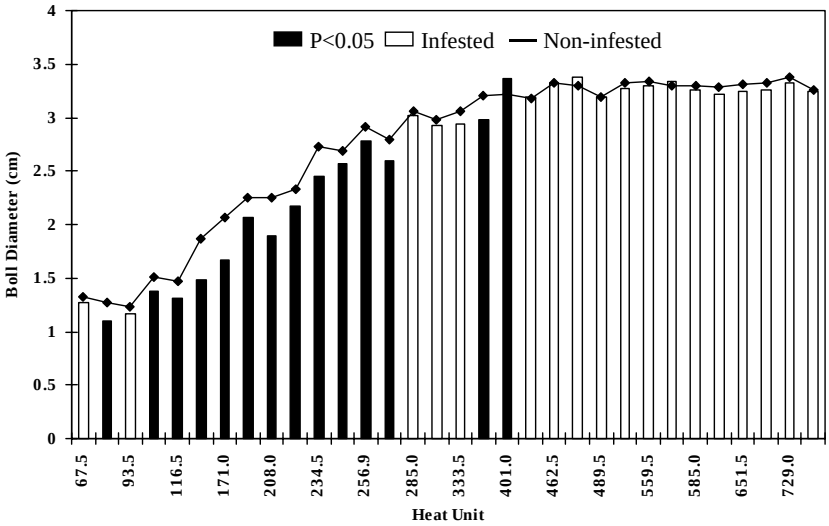

Figure 2.6. Relationship of heat unit accumulation and brown stink bug injury on boll diameter of individual bolls (bars represent mean diameters of infested bolls and the line represents mean diameters of non-infested bolls; dark bars represent diameters of infested bolls significantly [α = 0.05] different from non-infested boll diameters [$P < 0.05$]), 2002.

elongation is greatest. As growth continued, brown stink bug appeared to hinder boll development. Bolls infested with brown stink bug attained maximum size 29.4 heat units before non-infested bolls, resulting in a 0.03 cm decrease in diameter.

Results from field studies demonstrated that infestations of brown stink bug and southern green stink bug adults on cotton seedlings (pre-squaring), cotton with a small (match-head) square, and individual large (pre-candle) squares did not negatively affect growth and development of cotton, and production of bolls from squares. Persistent infestations of third instar and fourth-fifth instar southern green stink bug may have the ability to induce abscission or injure squares. If adults immigrate into cotton fields early in the season and oviposit, the subsequent immatures are forced to feed on pre-flowering cotton. Nymphs do not have the ability to emmigrate to other hosts; therefore, squares would be the structure most likely to incur injury. During this period, other pests including tarnished plant bugs and thrips are more likely to induce square abscission.

Brown stink bug adults are capable of significantly injuring cotton when bolls are present. These data are similar to that for other stink bug species and life stages. In these studies, brown stink bug-induced abscission in bolls that accumulated ≤ 350 heat units beyond anthesis (ca. 14 d beyond anthesis). Bolls infested with brown stink bug that accumulated > 351 heat units beyond anthesis, even if fed upon, did not abscise from the plant. During early boll development (through 266.5 heat units beyond anthesis), injury may occur as reductions in diameter during the period when fiber elongation is most rapid. Bolls infested with brown stink bug produced significantly fewer harvestable carpels per boll (≤ 400 heat units) and lower seedcotton yields (≤ 550 heat units). Seed harvested from bolls previously infested with brown stink bug had reduced germination in bolls ≤ 600 heat units beyond anthesis.

The results from these studies should provide a better understanding of the susceptibility of selected fruiting forms to stink bug injury and to define those periods in which to intensively scout for stink bugs in cotton fields. Therefore, these studies indicate control measures for stink bugs should be initiated at the time plants begin to set bolls. Small bolls are very sensitive to abscission. However, larger bolls that are injured by stink bugs remain on the plant. No injury to bolls occurred when a boll accumulated ≥ 600 heat units beyond anthesis (ca. 24 d beyond anthesis). The studies did not demonstrate significant injury to cotton prior to flowering. Injury to pre-flowering cotton could potentially occur if infestations of nymphs persist over long periods of time or if cotton is the only available host for adults to reproduce.

References Cited

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Adamczyk, J.J., V.J. Mascarenhas, G.E. Church, B.R. Leonard, and J.B. Graves. 1998. Susceptibility of conventional and transgenic cotton bolls expressing the *Bacillus thuringiensis* CryIA(c) δ-endotoxin to fall armyworm (Lepidoptera: Noctuidae) and beet armyworm (Lepidoptera: Noctuidae) injury. J. Agric. Entomol. 15: 163-171.

Association of Official Seed Analysts (AOSA). 2000. Rules for seed testing. AOSA, Las Cruces, NM.

Bagwell, R.D. and N.P. Tugwell. 1992. Defining the period of boll susceptibility to insect damage in heat-units from flower, pp. 767-768. *In* D. Herbert and D. Richter [eds.], Proc. 1992 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Barbour, K.S., J.R. Bradley, and J.S. Bachelor. 1990. Reduction in yield and quality of cotton damaged by green stink bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 83: 842-845.

Burris, E., J.H. Pankey, B.R. Leonard, and J.B. Graves. 1997. Tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), in cotton. Research Information Sheet 101. LSU AgCenter.

Cothren, J.T. 1999. Physiology of the cotton plant, pp. 207-268. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Fromme, D.D. 2000. The effect of boll age on stink bug feeding, boll damage and yield loss, p. 10. Cotton Incorporated Research Summary. Cotton Incorporated, Raleigh, NC.

Greene, J.K. and G.A. Herzog. 1999. Management of stink bugs using symptoms of boll injury as a monitoring tool, pp. 1041-1044. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council. Memphis, TN.

Guinn, G. 1986. Hormonal relations during reproduction, pp. 113-136. *In* J.R. Mauney and J.M. Stewart [eds.], Cotton physiology. The Cotton Foundation, Memphis, TN.

Hanny, B.W., T.C. Cleveland, and W.R. Meredith. 1977. Effects of tarnished plant bug, *Lygus lineolaris*, infestations on presquaring cotton, *Gossypium hirsutum*. Environ. Entomol. 6: 460-462.

Layton, M.B. 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. Southwestern Entomol. Supp. No. 23: 7-20.

Leffler, H.R. 1976. Development of cotton fruit I: accumulation and distribution of dry matter. Agron. J. 68: 855-857.

Leonard, B.R., J.B. Graves, and P.C. Ellsworth. 1999. Insect and mite pests of cotton, pp. 489-552. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Oosterhuis, D.M. and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant, pp. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Pack, T.M. and P. Tugwell. 1976. Clouded and tarnished plant bugs in cotton: a comparison of injury symptoms on fruit parts. Arkansas Agricultural Experiment Station Report Series No. 226.

Roberts, P. 1999. Observations of emerging pests in low spray environments, p. 1034. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN

Russell, J.S. 1999. Effects of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feeding on cotton boll abscission and yield. M.S. thesis, Louisiana State University, Baton Rouge. 47 pp.

SAS Institute. 1998. SAS User's manual, version 8. SAS Institute, Cary, NC.

Schubert, A.M., C.R. Benedict, J.D. Berlin, and R.J. Kohel. 1973. Cotton fiber elongation-kinetics of cell elongation and secondary wall thickening. Crop Sci. 13: 704-709.

Todd, J.W. 1989. Ecology and behavior of *Nezara viridula*. Annu. Rev. Entomol. 34: 273-292.

Toscano, N.C. and V.N. Stern. 1976. Cotton yield and quality loss caused by various levels of stink bug infestations. J. Econ. Entomol. 69: 53-56.

Tugwell, P., S.C. Young, B.A. Dumas, and J.R. Phillips. 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury, and significance of wild hosts. Arkansas Agricultural Experiment Station Report Series 227.

Wene, G.P. and L.W. Sheets. 1964a. Lygus bug injury to presquaring cotton. University of Arizona Agricultural Experiment Station. Tech. Bull. No. 166.

1964b. Notes on control of stink bugs affecting cotton in Arizona. J. Econ. Entomol. 57: 60-62.

Williams, M.R. 2002. Cotton insect losses 2001. http://www. msstate.edu/ Entomology/ CTNLOSS/ 2001/ 2001loss.htm.

CHAPTER 3

BOLL INJURY AND YIELD LOSSES IN COTTON ASSOCIATED WITH BROWN STINK BUG DURING FLOWERING

Introduction

A complex of stink bugs, including brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say), are most injurious to cotton, *Gossypium hirsutum* L., during boll development stages. Feeding by stink bugs can cause small bolls to abscise, reduce lint quality, seed germination, and overall yield (Chapter 2, Wene and Sheets 1964, Barbour et al. 1990, Greene et al. 1999). Stink bugs do not significantly injure cotton seedlings or flower buds (squares) during pre-flowering growth stages (Chapter 2).

Thresholds for timing insecticide treatments against stink bugs in cotton have been established across the mid-southern and southeastern United States. These thresholds rely on methods that estimate stink bug densities [1 adult or nymph/1.8 row-m (1 adult or nymph/6 row-feet), or 1 adult or nymph/25 sweeps] or stink bug-injured bolls (Patrick and Lentz 2001, Anonymous 2002, Bagwell et al. 2002, Boyd and Phipps 2003, Johnson et al. 2002, Roberts et al. 2003, Bachelor and Van Duyn 2003). Although sweep nets and shake sheets are valuable tools for detecting stink bug infestations in other row crops, these tools can be inefficient in detecting infestations in cotton because of tall, dense vegetative growth (Willrich et al. 2003). Examining bolls for internal feeding symptoms [the presence of wart-like callous tissue or punctures (water-soaked lesions) on the internal carpel wall, with or without stained lint] has been an effective monitoring tool (Bundy et al. 2000, Greene and Herzog 1999). Current recommendations based on this protocol vary on the specific boll size sampled [1/3-size, medium-size, quarter-size (2.426 cm diameter), thumb-size, or 12 to 16 d old bolls] and on the

boll injury level (10-20%) that triggers a treatment (Patrick and Lentz 2001, Anonymous 2002, Bagwell et al. 2002, Johnson et al. 2002, Boyd and Phipps 2003, Bachelor and Van Duyn 2003, Roberts et al. 2003).

Cotton has an indeterminate growth habit in which vegetative and reproductive growth occur simultaneously (Oosterhuis and Jernstedt 1999). The interval of time between production of flowers at the same relative position on a successively higher sympodial branch is ca. 3 d (vertical fruiting interval); whereas, the development of two flowers on the same sympodial branch at successive positions is ca. 6 d (horizontal fruiting interval) (Oosterhuis and Jernstedt 1999). Thus, as the cotton plant matures, various boll ages will be present and the total quantity of bolls will increase.

The following study was conducted to determine the relationship among boll density, total boll injury, percent injury during defined periods of flowering, and the influence of these factors on seedcotton yield. These data can be used to improve sampling protocols, including action thresholds, that rely on presence of injury in bolls to initiate treatments for stink bugs in cotton.

Materials and Methods

Study Site and Experimental Design

These studies were conducted at the Macon Ridge Research Station near Winnsboro, Louisiana (Franklin Parish) during 2002 and 2003. The soil at the site was a Gigger-Gilbert silt loam complex. The field plots were planted to 'DP458BR' on 23 May 2002 and 30 Apr 2003. General agronomic practices for optimum fertilization and pest control were followed as recommended by the LSU AgCenter. Supplemental irrigation was applied to all plots on an as-

needed basis. All non-target pests were suppressed with weekly applications of insecticides at recommended rates.

Plot size was two rows (101.6 cm row centers) x 3.3 m in length. Plant densities were thinned to nine plants per m within three weeks after plant emergence. Treatments were placed in a randomized complete block design with a 5 x 2 factorial treatment arrangement in four replications. The first factor consisted of stink bug-infested (30 adults/cage) and non-infested plots. Brown stink bug adults were collected from soybean, *Glycine max* (L.) Merrill, and held for ca. 24-h prior to infestation during each week as described in Chapter 2. Translucent cages (32 nylon mesh/linear cm, Synthetic Industries, Greenville, Georgia) were placed over each plot containing the stink bugs.

The second factor consisted of five consecutive 7 d flowering intervals. The intervals corresponded to each of the initial five weeks of flowering. The first week of flowering was defined as 50% of plants in each plot across the test area with ≥ 1 flower or boll (22 Jul 2002 and 7 Jul 2003). Subsequent infestations occurred every 7 d following the first week of flowering for a total of five unique infestation intervals. During each week, the growth stage of the plants within the study site was recorded as number of main stem nodes above a sympodial branch with a flower on the first node (NAWF). In 2002, the NAWF growth stage during weeks one, two, three, four, and five was 7-9, 6-8, 5-6, 3-4, and <4, respectively, and in 2003 was 8-10, 7-9, 5-8, 4-6, and <3, respectively.

Evaluation and Analysis of Boll Injury

All green bolls from one row of each infested and non-infested plot were removed at the completion of each pre-determined flowering interval, within ca. 48 h of removal of cages. Bolls were transported to the laboratory, and stored in chilled coolers and refrigerators until inspected

for injury. Multiple dependent variables related to injury were obtained for each green boll harvested. Symptoms of injury recorded for each boll included the total number of carpels (locules) within each boll that possessed at least one wart (callous tissue) or puncture (water-soaked lesion) on the internal carpel wall; the total number of carpels within each boll that possessed discolored lint corresponding to a wart or puncture on the internal carpel wall; and the total number of carpels within each boll with external symptoms (dark, circular indentations) on the carpel wall that corresponded to a wart or puncture on the internal carpel wall.

Four categories were used to describe boll injury by stink bugs. Within each infested and non-infested plot, the percentage of bolls with at least one carpel per boll with internal injury (single carpel injury), bolls with at least two carpels per boll with internal injury (multiple carpel injury), bolls with at least one carpel per boll with internal injury and lint discoloration (lint discoloration), and bolls with at least one carpel per boll with internal injury and external symptoms (external injury). Paired t-tests were used to compare categories of injury between the infested and non-infested treatment in each week (PROC TTEST, SAS Institute 1998). Contrast analysis within the infested treatment were used to make comparisons between 'single carpel injury and multiple carpel injury', 'single carpel injury and lint discoloration', and 'single carpel injury and external injury' (PROC GLM, SAS Institute 1998).

Boll injury data for infested plots was corrected for injury within non-infested plots using Abbott's formula (Abbott 1925): {[(% injury in infested treatment)–(% injury in non-infested treatment)]/[100-% injury in non-infested treatment]} x 100. Analysis of variance (ANOVA) was used to compare corrected injury, total bolls present, and total bolls injured among weeks. A general linear model was used, with week as the independent variable (PROC GLM, SAS

1998). The Fisher protected least significant difference (LSD) test was used for mean separation ($\alpha = 0.05$).

Seedcotton Yield

Seedcotton yield was harvested from each plot on the second row. During weeks one, two, three, and four, "snap-on-tags" (A.M. Leonard, Piqua, OH) were placed on each plant of the second row on the main stem node below the sympodial branch possessing a flower on the first node at the time cages were removed from plots. Each plant was tagged to separate those bolls exposed to stink bugs (exposed canopy) and those bolls produced by the plant after stink bugs were removed (non-exposed canopy) (Figure 3.1). The exposed and non-exposed canopy on plants were not separated in week five because all bolls on plants were exposed to stink bugs.

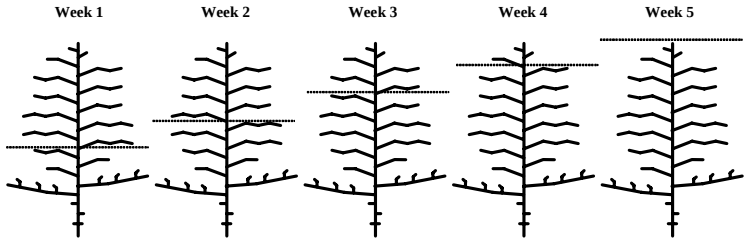

Figure 3.1. Diagram of a cotton plant illustrating fruiting branches present during time of infestation. All bolls below the dashed line were available for stink bug injury. Bolls above the dashed line were not present and therefore not exposed to stink bugs across the initial five weeks of flowering.

Plots were chemically defoliated and hand-harvested. Mean seedcotton yield in the exposed canopy, non-exposed canopy, and total canopy (exposed and non-exposed canopy) was compared between the infested and non-infested treatment. Data were subjected to analysis of covariance because of differences in boll number between treatments (PROC GLM, SAS Institute 1998). Data for 2002 and 2003 were analyzed independently because environmental conditions differed between years.

Results

In 2002 and 2003, significantly more bolls in the stink bug-infested treatment exhibited single carpel injury, multiple carpel injury, and lint discoloration as compared to the non-infested treatment during each week ($P < 0.05$) (Table 3.1). The percentage of bolls exhibiting external injury in the stink bug-infested treatment was significantly greater than that in the non-infested treatment during 2002 in weeks two, three, four, and five and during 2003 in week four ($P < 0.05$).

During each week for both years there was no significant difference between the percentage of bolls with single carpel injury and bolls with lint discoloration in the stink bug-infested treatment ($P > 0.05$) (Table 3.1). In contrast, the proportion of bolls with single carpel injury was significantly greater than bolls with multiple carpel injury across all weeks ($P < 0.05$). Bolls with single carpel injury in the infested treatment was significantly greater ($P < 0.05$) than bolls with external injury for each week during both years.

In 2002, bolls expressing single locule injury was significantly different among weeks ($F = 4.05$, df = 4,12, $P = 0.0218$) (Table 3.2). Boll injury in the stink bug-infested treatment was significantly greater in week one, two, and five as compared to that in week four. Boll injury was not significantly different between weeks three and four. In 2003, boll injury was significantly different among weeks ($F = 6.14$, df = 4,12, $P = 0.0090$). Boll injury was significantly greater in week two as compared to weeks three, four, and five. There were no significant differences in boll injury between weeks one and two.

There was a significant difference in boll density during 2002 ($F = 91.2$, df = 4,12, $P = 0.0001$) and 2003 ($F = 43.37$, df = 4,12, $P = 0.0001$) (Figure 3.2). Boll number ranged from 47.3 to 311.0 during weeks one through five in 2002. Boll density significantly increased in each

Table 3.1. Brown stink bug-induced boll injury for the total boll population, 2002 and 2003.

			Boll Injury (%) / *P*-value[1]							
Year	Week	Treatment	Single carpel[2]		Multiple carpel[3]		Lint Discoloration[4]		External Injury[5]	
2002	1	Infested	29.3		21.1		24.4*[6]		7.4	
		Non-Infested	7.7	0.0143	2.7	0.0178	6.8	0.0187	2.3	0.2990
	2	Infested	30.4		19.4		25.9*		7.7	
		Non-Infested	5.6	0.0002	1.7	0.0008	3.0	0.0015	1.1	0.0496
	3	Infested	16.0		7.8		14.3*		3.3	
		Non-Infested	3.2	0.0033	1.1	0.0036	2.9	0.0040	0.6	0.0371
	4	Infested	16.5		6.8		15.7*		3.8	
		Non-Infested	6.4	0.0059	0.9	0.0003	5.0	0.0026	0.9	0.0024
	5	Infested	25.4		15.0		25.3*		8.7	
		Non-Infested	6.8	0.0001	1.5	0.0012	6.8	0.0001	1.2	0.0001
2003	1	Infested	16.1		9.1		14.9*		3.7	
		Non-Infested	3.6	0.0010	0.0	0.0147	3.3	0.0006	5.0	0.2745
	2	Infested	18.0		10.5		17.4*		5.4	
		Non-Infested	2.4	0.0004	0.7	0.0007	2.4	0.0003	3.4	0.2612
	3	Infested	12.1		5.8		11.7*		3.7	
		Non-Infested	3.3	0.0031	0.6	0.0044	3.3	0.0213	3.6	0.9646
	4	Infested	17.3		10.2		16.3*		0.5	
		Non-Infested	6.4	0.0015	0.9	0.0013	6.2	0.0024	1.4	0.0282
	5	Infested	17.8		8.8		15.0*		1.7	
		Non-Infested	6.9	0.0161	2.4	0.0121	6.2	0.0203	2.4	0.5403

[1]Infested and non-infested means compared within each column and week ($P < 0.05$).
[2] ≥ 1 carpel per boll with internal injury (wart or puncture).
[3] ≥ 2 carpels per boll with internal injury.
[4] ≥ 1 carpel per boll with internal injury and lint discoloration.
[5] ≥ 1 carpel per boll with internal injury and exocarp symptoms.
[6]All means within rows of the infested treatment are compared to single carpel injury. Values with asterisks are not significantly different from single carpel injury ($P > 0.05$).

Table 3.2. Boll injury induced by brown stink bug during each week of flowering.

	Boll Injury (%)[1,2]	
Week	2002	2003
1	22.0ab	12.9ab
2	27.4a	16.0a
3	13.6bc	9.2b
4	10.7c	11.6b
5	20.0ab	11.5b
P-value	0.0218	0.0090

Column means followed by the same letter are not significantly different (LSD, $P > 0.05$).
[1] ≥ 1 carpel per boll with a wart (callous tissue) or puncture (water-soaked lesion) on the internal carpel wall.
[2]Corrected for injury within the non-infested treatment.

week through week four; however, there was no significant increase in boll number from that in week four to week five. Boll density ranged from 67.8 to 348.0 during week one through five in 2003. Significant increases in boll density were observed in each of the initial three weeks. Number of bolls was not significantly different between week three and four. In week five, boll density was significantly greater than in other weeks.

The number of bolls injured was significantly different among weeks during 2002 (F = 5.44, df = 4,12, P = 0.0065) and 2003 (F = 4.66, df = 4,12, P = 0.0121) (Figure 3.2). In both years, significantly more bolls were injured in week four and five as compared to week one. There was no significant difference in the number of bolls injured among weeks one, two, and three. Boll injury observed in weeks two and three was also not significantly different from that observed in week four.

Brown stink bug injury significantly influenced seedcotton yields (Table 3.3). In 2002, infestations during weeks one and two significantly reduced seedcotton harvested from the exposed canopy (boll cohort exposed to stink bugs) in the infested treatment compared to that in

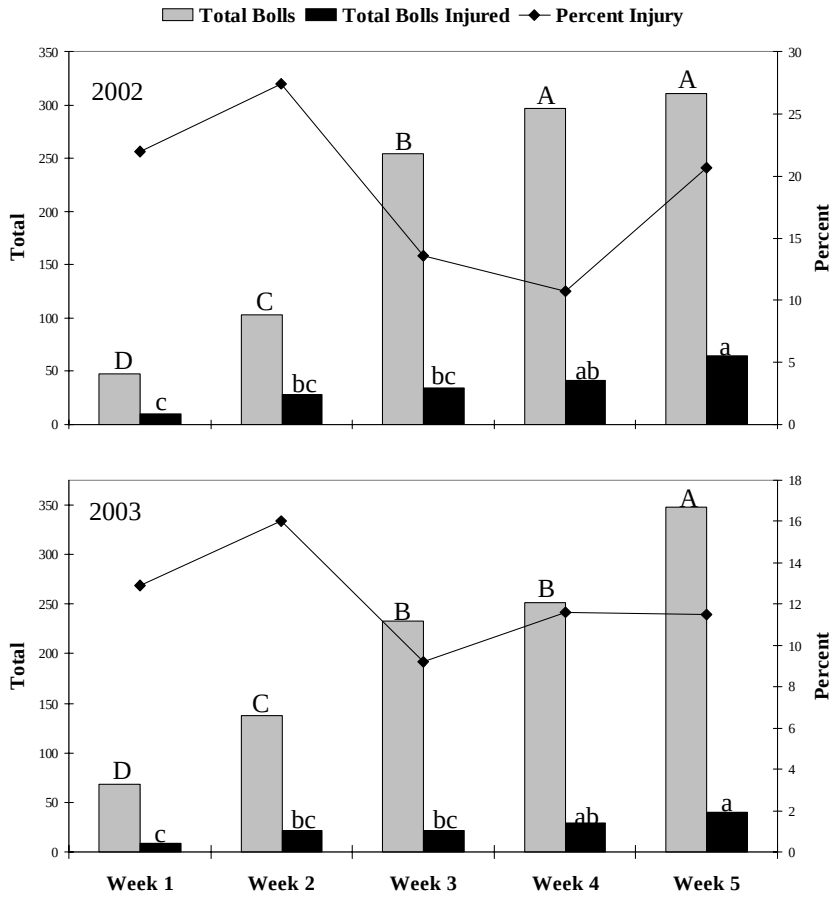

Figure 3.2. Relationship between boll density, number of injured bolls, and percent injured bolls as influenced by brown stink bug infestations, 2002 and 2003. Total and percent injury in the infested treatment is corrected for injury within the non-infested treatment. Bars followed by the same capital or lower-case letter are not significantly different.

the non-infested treatment. Seedcotton harvested from non-exposed bolls for week one of the infested treatment was significantly greater than that harvested from the non-infested treatment. Yield from non-exposed canopy between the infested and non-infested treatment was not significantly different during week two. Total seedcotton yields were not significantly reduced by brown stink bug for weeks one and two. Stink bug infestations during weeks three and four did not significantly affect seedcotton yield in the exposed, non-exposed, or total canopy. Total (exposed canopy) seedcotton harvested from the infested treatment during week five was

Table 3.3. Influence of brown stink bug infestations on seedcotton yield, 2002 and 2003.

			Seedcotton Yield (g / 3.3 row-m)					
			Exposed Canopy		Non-Exposed Canopy		Total Canopy	
Year	Week	Treatment	Yield	*P*-value	Yield	*P*-value	Yield	*P*-value
2002	1	Infested	917.2		975.4		1895.6	
		Non-Infested	1131.4	0.0200	877.4	0.0411	2005.7	0.1171
	2	Infested	838.1		735.9		1593.2	
		Non-Infested	1003.5	0.0173	678.8	0.0839	1663.1	0.2302
	3	Infested	1116.5		425.5		1521.7	
		Non-Infested	1154.0	0.3508	370.0	0.2069	1544.3	0.3916
	4	Infested	1278.0		106.1		1378.2	
		Non-Infested	1365.6	0.1361	104.2	0.4056	1475.7	0.1091
	5	Infested	1028.5		-----		1028.5	
		Non-Infested	1121.7	0.0251	-----	-----	1121.7	0.0251
2003	1	Infested	532.0		1353.3		1884.7	
		Non-Infested	513.5	0.5108	1333.9	0.8629	1848.1	0.7515
	2	Infested	946.5		661.4		1627.7	
		Non-Infested	941.6	0.9379	722.6	0.0540	1644.5	0.8721
	3	Infested	1041.4		270.4		1344.3	
		Non-Infested	1319.5	0.1338	269.9	0.9760	1556.9	0.2479
	4	Infested	979.3		75.4		1051.5	
		Non-Infested	1289.9	0.0258	94.2	0.2499	1387.2	0.0317
	5	Infested	1174.9		-----		1174.9	
		Non-Infested	1291.8	0.0470	-----	-----	1291.8	0.0470

[1]*P*-values represent comparisons between treatments within a week ($P < 0.05$).
[2]Seedcotton yield from bolls exposed to stink bugs.
[3]Seedcotton yield from bolls not exposed to stink bugs.
[4]Exposed and non-exposed canopy yields.

significantly less than that in the non-infested treatment.

In 2003, there was no significant difference between the infested or non-infested treatment for seedcotton yield harvested from the exposed, non-exposed, or total canopy following stink bug infestations in weeks one, two, and three (Table 3.3). Stink bug infestations

during weeks four and five resulted in significantly less seedcotton harvested from the exposed and total canopy. There was no significant difference in non-exposed canopy seedcotton yield between treatments during week four.

Discussion

Brown stink bugs are capable of causing significant boll injury at a density of 4.6 adults/row-m. The density of stink bugs per cage in our studies was 8.2-fold greater than the level (1 stink bug/1.8 row-m) currently recommended for initiating insecticide treatments against stink bugs in cotton across several states in the mid-south and southeastern United States. These infestation densities were used to facilitate the occurrence of injury and document that brown stink bugs are capable of inducing cotton yield losses.

All plots received frequent applications of an insecticide for control of native cotton pests, but injury in the non-infested treatment ranged from 0.0 to 7.7% across both years. This injury may have been caused by the immigration of other stink bugs or tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), into the study area between insecticide applications. Tarnished plant bugs are capable of causing internal injury indistinguishable from that of stink bugs (Greene et al. 1999). Internal injury on carpel walls has been documented in bolls ≤ 12 d old (ca. 250 heat units beyond anthesis); however, tarnished plant bugs do not penetrate or cause yield loss in bolls > 12 d old (Greene et al. 1999, Russell 1999, Horn et al. 1999). Therefore, in our studies, most boll injury should be associated with stink bug infestations.

Lint discoloration was associated more with internal injury to carpels (warts or punctures) than with external symptoms of stink bug feeding. These results strongly support those of Bundy et al. (2000), in which ca. 20% of bolls damaged by brown stink bug, southern green stink bug, or green stink bug displayed internal feeding symptoms but lacked evidence of

feeding symptoms on the exterior boll wall. External symptoms significantly underestimate total stink bug-injured bolls in a cotton field. Sampling methodologies that rely on opening bolls for injury, rather than examining the outer surface of bolls for external symptoms, should be a stronger indicator of stink bug presence in a cotton field. In our study during 2002, the weeks (one, two, and five) in which seedcotton yields were lower for exposed bolls, there was also ca. 2-fold increase in bolls with evidence of exocarp feeding as compared to weeks (three and four) in which seedcotton yields were not affected. This relationship between external injury and yield loss was not evident during 2003. The presence of stink bug feeding symptoms on the boll exocarp and relationship to yield loss was inconsistent in our studies.

The proportion of bolls displaying stink bug injury in at least one locule was significantly greater than for bolls displaying injury in multiple carpels. Stink bugs appear to puncture many bolls rather than sustain feeding on a small cohort of bolls. This indicates stink bug injury should occur in proportions greater than that for actual stink bug density. Current action thresholds define an injured boll as having ≥ 1 injured carpel. Therefore, if boll yields are not significantly reduced by single injury, then sampling protocols should consider multiple locule injury as a criteria for defining boll injury. Further studies should examine the relationship of single and multiple injury to individual boll yields.

Generally, boll injury was greater in week one and two as compared to other weeks, with exception for week five in 2002. The number of bolls available to stink bugs increased on plants as the flowering period progressed through the five weeks. There was a 2.4 and 1.8-fold increase during 2002 and 2003, respectively, in boll density between week two and three. Therefore, boll injury was greater in weeks one and two because the difference between total

bolls and stink bug-injured bolls was smaller compared to other weeks in which boll density was high. Fewer total bolls were injured in weeks one and two in these studies.

In week five during 2002, boll injury was similar to that observed during weeks one and two. A high percentage of boll injury may be explained by stink bug infestations concentrated in the upper portion of the canopy and feeding on a preferred cohort of bolls. Other studies have indicated bolls accumulating ca. 165 through 495 heat units beyond anthesis were more commonly injured by brown stink bug in week five of flowering (Chapter 4). Bolls within these age cohorts were present in the upper portion of the plant canopy. Stink bugs may have been concentrated in this portion of the canopy, spending less time searching, but injuring more bolls. During 2003, boll injury in week five was less than week one and two. In this year, total bolls available from the boll cohort accumulating ca. 165 through 495 heat units beyond anthesis was 1.6-fold greater than that available during 2002 (Chapter 4). Lower boll density in 2002 likely accounted for the greater boll injury in week five compared to 2003, when the density of stink bugs remained constant.

In both years, lower seedcotton yields for bolls exposed to stink bugs could have resulted from boll abscission. In these studies, boll abscission that occurred during each week of flowering was not measured. Injury by brown stink bug was based upon bolls that remained on plants after each week of infestation. However, several stink bug species can cause boll abscission (Wene and Sheets 1964, Barbour et al. 1990). Brown stink bug are capable of inducing abscission in bolls that have accumulated through 350 heat units beyond anthesis (Chapter 2). Also, lower seedcotton yields in the canopy exposed to stink bugs could have resulted from reduced individual boll weights. In no-choice feeding studies, brown stink bug adults and southern green stink bug nymphs reduced seedcotton yield for individual bolls that

have accumulated ≤ 550 heat units beyond anthesis (ca. 22 d) and ≤ 472 heat units (ca. 21 d) beyond anthesis, respectively (Chapter 2, Greene et al. 2001). Infestations of *Euschistus conspersus* Uhler on whole-plants significantly reduced seedcotton yield by 2.5 and 1.4-fold compared to the non-infested treatment at level of 8 stink bugs/plant for 7 d and 100 stink bugs/98 plants for 100 d, respectively (Toscano and Stern 1976).

Higher seedcotton yields in the non-exposed canopy of the stink bug-infested treatments was a result of the cotton plant's ability to compensate for boll losses or injury to individual bolls that occurred in the initial weeks of flowering. In these studies, when compensation in the non-exposed canopy occurred, total yield was not significantly decreased following infestations in week one through four in 2002 and in weeks one through three in 2003. Removal of stink bugs early in the flowering period provided ample time for the cotton plant to produce more fruit or re-allocate photosynthates to other developing bolls. In contrast, infestations occurring during week four in 2002 and during weeks four and five in 2003, resulted in total seedcotton yield that was significantly less than the non-infested treatment. These infestations were sufficiently late in the flowering period that cotton plants did not have time to produce more bolls or larger individual bolls.

The ability of cotton to compensate for fruit loss from insects during early reproductive growth stages has been widely documented. Compensation can occur through retention of existing flowers, production of additional flowers on more distal positions of sympodial branches, production of more flowers on monopodial (vegetative) branches, or production of additional main stem nodes with sympodial branches (and thus more fruiting sites) (Heitholt 1999). Jones et al. (1996) demonstrated early flower removal (third week and earlier) did not significantly affect seedcotton yield; whereas, later flower removal (fourth week and beyond)

resulted in significantly lower yields. Increased yields in other studies have been associated with production and retention of bolls on fruiting sites further away from the mainstem (Mulrooney et al. 1992, Jones et al. 1996). Other studies have demonstrated individual boll weights have been increased as number of bolls per plant decreased, either by hand-removal or injury from insects (Brook et al. 1992). Boll production, retention, and weight gain are likely mechanisms for the yield compensation observed in the present study.

Compensatory reproductive development in cotton is not only a function of the duration of the recovery period, but on the environmental conditions. Compensation after fruit injury is facilitated if environmental factors allow for continued growth, flowering, and boll retention and if other plant stresses are managed (Dale 1959, Jones et al. 1996, Sadras 1996). These stresses can include high plant density, low soil fertility, low temperatures, or competition from pests (insects, weeds, pathogens) (Sadras 1996). Even when resource availability is high, boll dry weights have been significantly reduced (55-67%) when a limited period for optimum development is available for recovery from plant stressors (Sadras 1996).

The recommended sampling protocols that examine bolls for stink bug injury use an injury threshold ranging from 10-20%. In the present study during 2002, boll injury was greatest in weeks one, two, and five, and ranged from 20.0 to 27.4%. In weeks three and four, boll injury was significantly lower than other weeks, but did exceed the lower limit of the injury threshold. Seedcotton yields for bolls exposed to stink bugs was significantly reduced when boll injury was $\leq$ 20% (weeks one, two, and five). In contrast, no significant reductions in yield were observed in the canopy exposed to stink bugs when injury was < 15%. During 2003, the relationship between boll injury and yield from the exposed canopy was not similar to that in 2002. Boll injury was $\leq$ 16% in all weeks during 2003. Significantly less seedcotton was harvested from

the exposed canopy during weeks four and five. The least amount of boll injury also was observed in weeks three, four, and five. In both years, boll injury and yield data strongly suggest that ≤ 27.4% stink bug-injured bolls can occur through week three of flowering without significantly influencing final yields of cotton. This would be contingent on the ability of the cotton plant to compensate for boll injury. However, ≤ 20% injury in bolls exposed to stink bugs in weeks four and five, can result in reduced final yields. The significant impact of a relatively lower level of boll injury on yield is related to boll density. The density of bolls increases at a faster rate compared to the number of injured bolls; therefore, percent injury throughout the period of flowering decreases.

In this study, a correlation between boll injury and seedcotton yield was not obtained. Brown stink bug may not have injured a similar proportion of bolls between rows within a cage. Therefore, destructive sampling of one of the rows within the cage may have resulted in an over- or underestimation of the resulting seedcotton yield. Additionally, the microclimate within cages may have differed between rows.

The results from the present study are important for establishing sampling protocols that rely on boll injury as an indicator of stink bug presence. Brown stink bug injured more bolls as the flowering period progresses. There was a 6.2-fold and 4.6-fold increase from week one to five in the total number of bolls injured in 2002 and 2003, respectively. However, because boll density also increased on the plant, the percentage of bolls injured generally declined. Sampling protocols that employ a static injury level throughout the flowering period to initiate treatments against stink bugs may underestimate the potential impact of stink bug infestations. Based on the results from the present study, this may be particularly critical during the concluding periods of flowering when less injury can induce yield loss.

These data have shown as flowering progresses into weeks three, four, and five, it may be necessary to examine more bolls for injury in order to gain an accurate representation of the total boll population present in a cotton field at that time. Additionally, it would be necessary to sample more bolls if the objective of the sampling protocol is to have a static injury threshold. Future work should focus on the development of a dynamic injury level in which the initial injury levels would decrease as the season progresses and as the density of bolls increases. The goal of a sampling plan which employs a dynamic injury level would be to accurately determine boll injury for a field while examining a similar number of bolls as in a sampling plan that employs a static injury level.

These studies have demonstrated brown stink bug can injure bolls and reduce seedcotton yield during discrete periods of flowering. However, if growing conditions are optimal and other pests are managed appropriately, removal of stink bug infestations during the initial period of flowering may not affect final yield. Infestations occurring during the concluding 7 to 14 d of the flowering period will result in reduced yield because of the abbreviated period for the cotton plant to compensate for losses. Further studies are needed to determine the impact of continuous, rather than discrete, infestations of brown stink bug on cumulative injury and yield loss.

References Cited

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 2002. Cotton insect, disease, nematode, and weed control recommendations for 2002. Alabama Cooperative Extension Service. http://www.aces.edu/publications /anr/2002IPM/COTTON.02.PDF.

Bachelor, J.S. and J.W. Van Duyn. 2003. 2003 North Carolina agricultural chemical manual: insect control on cotton. http://ipm.ncsu.edu/agchem/chptr5/505.pdf.

Bagwell, R.D., Stewart, S., B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2002. Cotton Insect Control, 2002. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Barbour, K.S., J.R. Bradley, and J.S. Bachelor. 1990. Reduction in yield and quality of cotton damaged by green stink bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 83: 842-845.

Boyd, M.L. and B.J. Phipps. 2003. University of Missouri cotton insect management guide-2003. http://www.aes.missouri.edu/delta/muguide/ctnmgtgs.stinkbugs.

Brook, K.D., A.B. Hearn, and C.F. Kelly. 1992. Response of cotton, *Gossypium hirsutum* L., to damage by insect pests in Australia: manual simulation of damage. J. Econ. Entomol. 85: 1368-1377.

Bundy, C.S., R.M. McPherson, and G.A. Herzog. 2000. An examination of the external and internal signs of cotton boll damage by stink bugs (Heteroptera: Pentatomidae). J. Entomol. Sci. 35: 402-410**.**

Dale, J.E. 1959. Some effects of the continuous removal of floral buds on the growth of the cotton plant. Annals of Botany 23: 636-649.

Greene, J.K. and G.A. Herzog. 1999. Management of stink bugs using symptoms of boll injury as a monitoring tool, pp. 1041-1044. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Heitholt, J.J. 1999. Cotton: factors associated with assimilation capacity, flower production, boll set, and yield, pp. 235-269. *In* D.L. Smith and C. Hamel [eds.], Crop yield: physiology and processes. Springer-Verlag, Germany.

Horn, T.O., F.A Harris, J.T. Robbins, and R.E. Furr, Jr. 1999. Influence of boll age on susceptibility to tarnished plant bug injury, pp. 1044-1045. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Johnson, D.R., G.E. Studebaker, G. Lorenz, and J.K. Greene. 2002. Cotton insect management. University of Arkansas Cooperative Extension Service. http://www.uaex. Edu/other_areas/publications/PDF/FSA-2065.PDF.

Jones, M.A., R. Wells, and D.S. Guthrie. 1996. Cotton response to seasonal patterns of flower removal: I. Yield and fiber quality. Crop Sci. 36: 633-638.

Mulrooney, J.E., W.L. Parrott, and J.N. Jenkins. 1992. Response of cotton to damage by tobacco budworm (Lepidoptera: Noctuidae): changes in fruit distribution. J. Econ. Entomol. 85: 981-986.

Oosterhuis, D.M. and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant, pp. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Patrick, C.R. and G.L. Lentz. 2001. 2001 Cotton insect control guide. University of Tennessee Agricultural Extension Service. Pub No. PB387-1M-1/01.

Roberts, P.M., D. Jones, and J. Ruberson. 2003. 2003 Georgia pest management handbook. http://www.ent.uga.edu/pmh/Agronomic/Agronomic_Crops.htm.

Russell, J.S. 1999. Effects of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feeding on cotton boll abscission and yield. M.S. thesis, Louisiana State University, Baton Rouge, LA. 47 pp.

Sadras, V.O. 1996. Cotton compensatory growth after loss of reproductive organs as affected by availability of resources and duration of recovery period. Oecologia 106:432-439.

SAS Institute. 1998. SAS User's manual, version 8. SAS Institute, Cary, NC.

Toscano, N.C. and V.N. Stern. 1976. Cotton yield and quality loss caused by various levels of stink bug infestations. J. Econ. Entomol. 69: 53-56.

Wene, G.P. and L.W. Sheets. 1964. Notes on control of stink bugs affecting cotton in Arizona. J. Econ. Entomol. 57: 60-62.

Willrich, M.M., D.R. Cook, and B.R. Leonard. 2003. Sampling hemipteran pests in cotton: a challenge to cotton IPM, pp. 73-64. Proc. Fourth National Integrated Pest Management Symposium. Indianapolis, IN.

CHAPTER 4

DEFINING COTTON BOLL AGE COHORTS INJURED BY BROWN STINK BUG

Introduction

Brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say), are common pests of cotton in the mid-southern United States. Stink bugs are observed in cotton fields from seedling emergence until harvest. These insects have not been observed to significantly injure cotton seedlings or flower buds (squares) during pre-flowering growth stages (Chapter 2). Stink bug infestations that occur during flowering can result in significant boll injury. Feeding by stink bugs can cause small bolls to abscise, and reduce lint quality, seed germination, and overall yield (Chapter 2, Wene and Sheets 1964, Barbour et al. 1990, Greene et al. 1999, Turnipseed et al. 2003). In no-choice feeding studies, brown stink bug adults and southern green stink bug nymphs reduced seedcotton yield in bolls that accumulated ≤ 550 heat units (ca. 22 d) and ≤ 472 heat units (ca. 21 d) beyond anthesis, respectively (Chapter 2, Greene et al. 2001).

In general, sampling and initiating treatments against stink bugs is difficult due to their mobility, in-field distribution, and host range (Willrich et al. 2003). For cotton, the problem is more complex than other crops because dense, tall canopies make using sweep nets and shake sheets a cumbersome task for estimating stink bug densities. Therefore, a sampling protocol that estimates boll injury rather than stink bug density for a cotton fields is being developed across the southeastern United States. Examining bolls for internal feeding symptoms [the presence of wart-like callous tissue or punctures (water-soaked lesions) on the internal carpel wall, with or without stained lint] is considered an effective monitoring tool (Bundy et al. 2000, Greene and Herzog 1999). Currently, recommendations based on this protocol vary on the size boll selected

to sample [one-third developed, medium-size, quarter-size (2.426 cm diameter), thumb-size, or 12 to 16 d old bolls] and on what percent injury (10-20%) constitutes a treatment (Patrick and Lentz 2001, Anonymous 2002, Bagwell et al. 2002, Johnson et al. 2002, Boyd and Phipps 2003, Bachelor and Van Duyn 2003, Roberts et al. 2003).

Data characterizing stink bug feeding preferences for bolls in various age classes (cohorts) is limited. The objective of the following study was to ascertain a boll age class that was most frequently injured by brown stink bugs. Determining the specific boll age and size could be used to refine sampling protocols such that only bolls within this range would be examined. Sampling bolls that are most likely to exhibit signs of injury during each week of flowering could improve a crop manager's sampling efficiency and decision-making confidence.

Materials and Methods

Studies were done at the Macon Ridge Research Station near Winnsboro, Louisiana (Franklin Parish) during 2002 and 2003. The soil was a Gigger-Gilbert silt loam complex. Field plots used for whole-plant infestations were planted to 'DP458BR' on 23 May 2002 and 30 Apr 2003. General agronomic practices for optimum fertilization and pest control were followed as recommended by the LSU AgCenter. Supplemental irrigation was applied to all plots on an as-needed basis. All non-target pests were suppressed with weekly applications of insecticides at recommended rates.

Plot size was two rows (101.6 cm row centers) x 3.3 m in length. Plant densities were thinned to nine plants per meter at three weeks after plant emergence. Treatments were replicated three times in a randomized complete block design with a 5 x 2 factorial treatment arrangement. The first factor consisted of stink bug-infested (30 adults/cage) or non-infested plots. Brown stink bug adults were collected from soybean, *Glycine max* (L.) Merrill, ca. 24-h

prior to infestation during each week, using a standard 15.0 cm diameter sweep net. Stink bugs were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan) to reduce mortality from physical injury and were fed a small quantity of washed green beans, *Phaseolus vulgaris* (L.).

The second factor consisted of five consecutive 7 d flowering intervals. The intervals corresponded to each of the initial five weeks of flowering. The first week of flowering was determined to be when 50% of plants in each plot across the test area had at least one flower or boll. Subsequent weeks occurred every 7 d following the first week of flowering. The growth stage of the plants within the study site was recorded as number of main stem nodes above a sympodial branch with a flower on the first node (NAWF). In 2002, the NAWF growth stage during weeks one, two, three, four, and five was 7-9, 6-8, 5-6, 3-4, and < 4, respectively, and in 2003 was 8-10, 7-9, 5-8, 4-6, and < 3, respectively.

All flowers on one row of each plot were marked with a yellow "snap-on-tag" (A.M. Leonard, Inc. Piqua, Ohio), placed on the peduncle (stem) between the flower and the fruiting branch. The date of anthesis was recorded on the tag to ascertain boll age upon removal from the plant. Boll age was calculated using heat unit accumulation beginning at anthesis, as described by Bagwell and Tugwell (1992). Heat units were calculated for each day of infestation as: [(maximum daily temperature + minimum daily temperature)/2]-15.5, where 15.5°C (60°F) is the minimum adequate temperature for cotton plant development. Flowers in the experimental plots were tagged prior to the initiation of week one until cages were removed at the completion of week five. Translucent cages (32 nylon mesh/linear cm, Synthetic Industries, Greenville, Georgia) were placed over each plot. All tagged, developing bolls from each infested and non-infested plot were removed at the completion of each week of flowering. Bolls were grouped

according to date of anthesis, transported to the laboratory, and stored in chilled coolers and refrigerators until inspected for injury.

For each green boll, the number of carpels (locules) with at least one wart (callous tissue) or puncture (water-soaked lesion) on the internal carpel wall was recorded. Individual bolls with at least one injured locule were classified as 'single locule injury'; whereas, bolls with at least two injured locules were classified as 'multiple locule injury'. Boll diameter was determined using a dial caliper (Forestry Suppliers, Inc., Jackson, MS). Individual boll measurements were taken at the widest diameter using two diametrically opposite points. Bolls were then placed into six discrete cohorts (Table 4.1). The intervals for each of the first five cohorts were calculated as: average number of heat units accumulated per day during the study x 7 d. In 2002, an average of 23.6 heat units were accumulated per day for a cohort interval of 165.2, whereas, in 2003, an average of 24.0 heat units were accumulated per day for a boll cohort interval of 168 heat units. A shorter interval in the sixth cohort during 2002 as compared to other cohorts is likely due to natural boll abscission that occurred during the first week of flowering.

Boll density was compared among cohorts in each week using analysis of variance (ANOVA) (PROC GLM, SAS 1998). Boll density of each cohort was then described as percent of total bolls tagged within that corresponding week. The percentage of bolls within each cohort that were injured (single and multiple locule injury) of the total bolls representing that cohort during each week and within infested and non-infested treatments was also determined. All injury data for infested plots was corrected for boll injury within non-infested plots based upon Abbott's formula (Abbott 1925): {[(% injury in infested treatment)–(% injury in non-infested treatment)]/[100-% injury in non-infested treatment]} x 100. Within each week, the interaction between corrected boll injury type (single locule injury and multiple locule injury) and boll

cohort was tested (ANOVA) (PROC GLM, SAS 1998). Corrected injury data were then subjected to ANOVA (PROC GLM, SAS 1998). A general linear model was used for each week and across all weeks to test for differences in boll injury among cohorts. The Fisher protected least significant difference (LSD) test was used for mean separation (α=0.05). Regression analysis was used to determine the relationship between boll cohort (across weeks) and diameter (PROC REG, SAS 1998). Data for 2002 and 2003 were analyzed independently because environmental conditions differed between years.

Results

During 2002, number of bolls in all cohorts during week one through five ranged from 40.2 to 200.4 (Table 4.1, Figure 4.1). Boll densities were significantly different among cohorts in week one (F = 37.5, df = 1,3, P = 0.0001), two (F = 32.8, df = 2,6, P = 0.0001), three (F = 44.9, df = 3,9, P = 0.0001), four (F = 30.4, df = 4,12, P = 0.0001), and five (F = 26.7, df = 5,15, P = 0.0001). Within week one, two, and three, boll density significantly decreased as boll cohort ages increased. However, in week three, there was no significant difference in boll density between cohorts two and three. In week four, boll densities were not significantly different between cohorts one and six, but were significantly less than that in other cohorts. Significant increases in boll density were observed in cohorts four, two, and three. In week five, boll density for cohorts three and four was significantly greater compared to other cohorts. Density of bolls for cohorts two and five was similar, and was significantly greater than boll densities in cohort one and six.

There was no significant interaction between boll cohort and boll injury type (single locule injury and multiple locule injury) during weeks one (F = 0.36, df = 1, 12 P = 0.5590), two (F = 0.16, df = 2,18, P = 0.8493), three (F = 0.52, df = 3,24, P = 0.6703), four (F = 0.16, df = 4,

Table 4.1. Boll density within cohort age-classes during the initial five weeks of flowering and their relationship to age (heat unit accumulation beyond anthesis) and diameter (cm)[1].

			Year / Week of Flowering				
			2002				
Cohort	Age[1]	Diameter	1	2	3	4	5
1	<165.2	0.607-2.014	30.9a	50.6a	78.1a	12.0d	8.0c
2	165.3-330.5	1.334-2.979	9.3b	21.5b	51.0b	47.5b	32.6b
3	330.6-495.8	2.184-3.322	-----	9.3c	41.8b	60.3a	61.9a
4	495.9-661.1	2.182-3.434	-----	-----	12.4c	34.3c	52.5a
5	661.2-826.4	2.100-3.421	-----	-----	-----	11.3d	36.8b
6	826.5-850.5	2.624-3.424	-----	-----	-----	-----	8.6c
			2003				
			1	2	3	4	5
1	<168	0.607-2.068	37.0a	56.3a	84.6a	32.1c	40.8b
2	169-336	1.161-3.134	23.0b	49.8a	84.0a	44.9b	21.5c
3	337-504	1.613-3.388	5.5c	26.5b	44.8b	86.9a	83.0a
4	505-672	1.935-3.586	-----	2.6c	20.4c	47.3b	92.3a
5	673-840	2.446-3.444	-----	-----	2.2d	16.6d	53.5b
6	841-1014	1.976-3.493	-----	-----	-----	2.5e	23.1c

Means within each year and column followed by the same letter are not significantly different ($P > 0.05$; LSD).
[1]Range of boll ages and diameters during the five weeks of flowering.

30, $P = 0.9587$), and five ($F = 0.43$, df = 5,6, $P = 0.8251$ in 2002. Therefore, injury data will be presented as single locule injury.

During week one, there were no significant differences in injury between boll cohorts one and two with means of 13.7 and 40.4%, respectively ($F = 6.65$; df = 1,3; $P = 0.2791$) (Figure 4.1). Significant differences in injury were observed among boll cohorts in weeks two

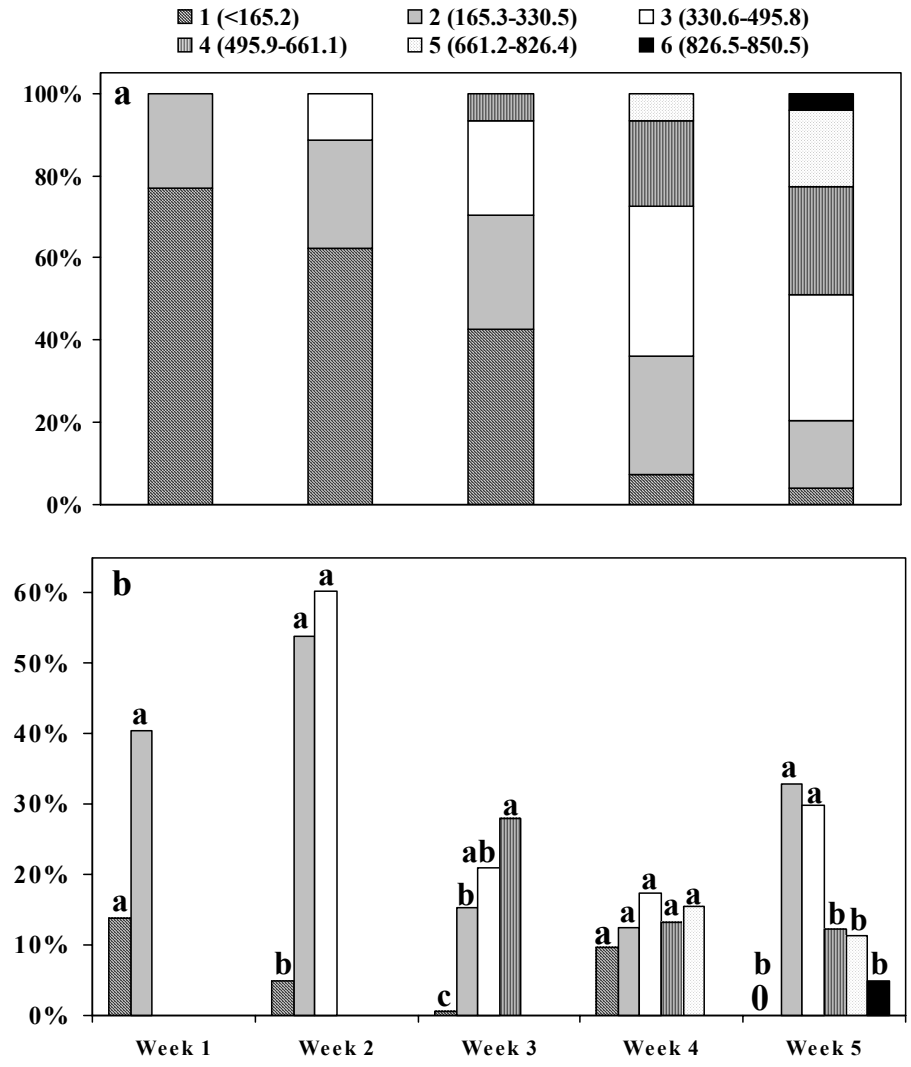

Figure 4.1. Percent distribution of boll cohorts (heat unit accumulation) (a) and percent brown stink bug injury within each cohort [bolls with ≥ 1 carpel with a wart or puncture] (b) during the initial five weeks of flowering, 2002. Injury for cohorts within weeks followed by the same letter are not significantly different ($P < 0.05$, LSD).

(F =16.97; df = 2,6; P = 0.0034) and three (F = 8.88; df = 3,9; P = 0.0214). In week two and three, brown stink bug injured significantly fewer bolls in cohort one compared to other cohorts. Boll injury for the oldest two cohorts (three and four) in week three was significantly greater than cohort one. In week four, there was no significant difference in the boll injury observed among cohorts (F = 0.50; df = 4,12; P = 0.7359). Boll injury across cohorts ranged from 9.6 to 17.4%. Significant differences in injury were observed among boll cohorts during week five (F

= 7.21; df = 5,15; $P = 0.0013$). Injury ranged from 0 to 32.8% and was significantly greater in cohorts two and three compared to all other cohorts.

During 2003, boll densities during week one through five ranged from 65.5 to 314.2 for all cohorts (Table 4.1, Figure 4.2). Boll densities were significantly different among cohorts in week one ($F = 38.0$; df = 2,6; $P = 0.0001$), two ($F = 19.0$; df = 3,7; $P = 0.0001$), three ($F = 78.0$; df = 4,11; $P = 0.0001$), four ($F = 347.7$; df = 5,14; $P = 0.0001$), and five ($F = 33.7$; df = 5,15; $P = 0.0001$). In the initial three weeks, boll densities significantly decreased as boll maturity increased. However, in weeks two and three, there was no significant difference in boll density between cohorts one and two. Boll density in cohort three during week four and cohorts three and four during week five were significantly greater than other cohorts. In week four, significant decreases in boll density compared to cohort three were observed in the following order of cohorts: two and four, one, five, and six. In week five, a significant decreases in boll density occurred from cohorts one and five to cohorts two and six.

There was no significant interaction between boll cohort and boll injury type (single locule injury and multiple locule injury) during weeks one ($F = 0.25$, df = 2,18, $P = 0.7812$), two ($F = 0.21$, df = 3,20, $P = 0.8909$), three ($F = 1.04$, df = 4,28, $P = 0.4047$), four ($F = 0.14$, df = 5,34, $P = 0.9816$), and five ($F = 0.13$, df = 5,36, $P = 0.9855$) during 2003. As in 2002, injury data will be presented as single locule injury.

During week one, boll injury was significantly different among cohorts ($F = 5.28$; df = 2,6; $P = 0.0475$) (Figure 4.2). Boll injury for cohort three was significantly greater than cohort one, with respective means of 44.2% and 0%. In week two, boll injury was significantly greater ($F = 6.29$; df = 3,7; $P = 0.0213$) within cohort four compared to cohort one and two, with

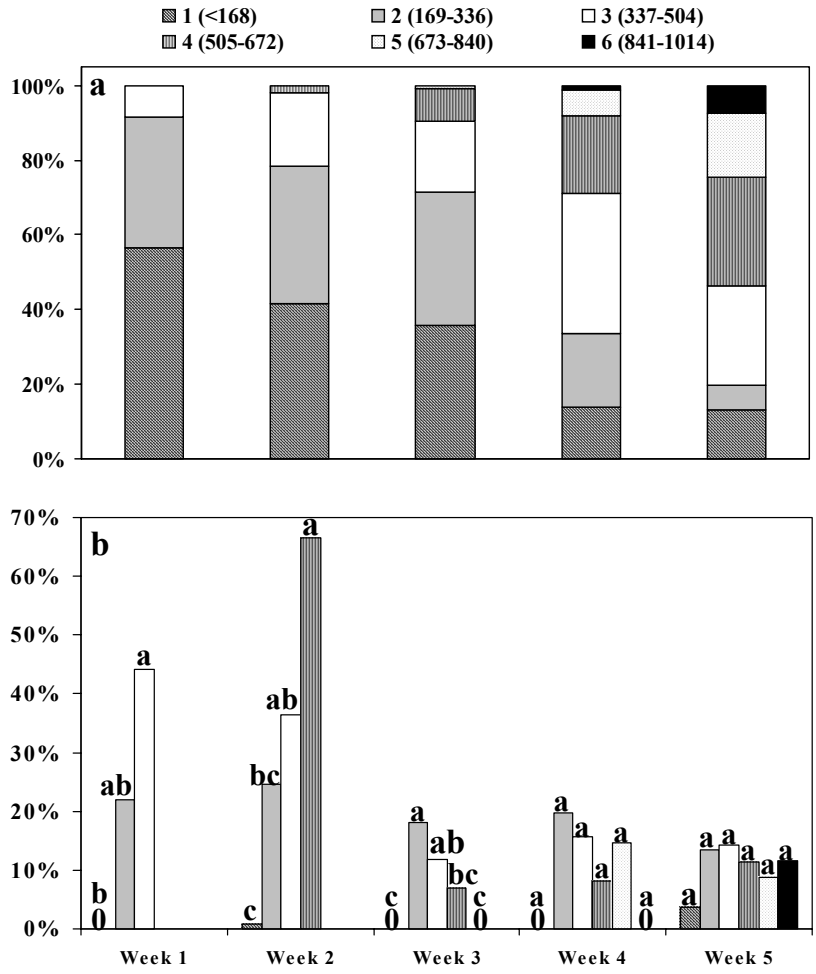

Figure 4.2. Percent distribution of boll cohorts (heat unit accumulation) (a) and percent brown stink bug injury within each cohort [bolls with ≥ 1 carpel with a wart or puncture] (b) during the initial five weeks of flowering, 2003. Injury for cohorts within weeks followed by the same letter are not significantly different ($P < 0.05$, LSD).

respective means of 66.5, 0.3, and 24.7%, respectively. In week three, boll injury was significantly different among cohorts (F= 8.65; df = 4,11; P = 0.0021). Injury within cohort two and three was significantly greater than that in cohort one and five. Boll injury among cohort one, four, and five was not significantly different, but was significantly less than cohort two. In week four, no significant difference in percent injury (0 to 19.8%) was observed among cohorts (F = 2.13; df = 5,14; P = 0.1233). Mean injury during week five ranged from 3.6 to 14.2% and did not differ among cohorts (F = 0.92; df = 5,15; P = 0.4926).

Across weeks, there were significant differences in the frequency of boll injury among cohorts during 2002 ($F = 5.69$; df = 5,71; $P = 0.0002$) and 2003 ($F = 6.34$; df = 5,83; $P = 0.0001$) (Figure 4.3). Across years, boll injury ranged from 0.8 to 13.4%, 16 to 18%, and 20 to 32%, for cohorts one, five, and six; cohort four; and cohorts two and three, respectively. There was a significant relationship describing boll diameter as a function of boll cohort in 2002 ($F = 53.59$; df = 2,3; $P = 0.0044$) and 2003 ($F = 63.56$; df = 2,3; $P = 0.0035$) (Figure 4.3). Boll diameters progressively increased for cohort one, two, and three; maintained constant diameter through cohort five; and declined slightly in cohort six.

Discussion

Total boll density increased through the initial five weeks of flowering. There was a 4.9 and 4.8-fold increase in boll number from week one to week five in 2002 and 2003, respectively. As flowering progressed through each week, new boll cohorts became available for stink bugs. The indeterminate growth habitat of cotton allows vegetative and reproductive growth to occur simultaneously after initiation of flowering (Oosterhuis and Jernstedt 1999). Flowers are produced at ca. 3 d intervals at the same relative position on a successively higher fruiting branch and at ca. 6 d intervals at adjacent sites on the same fruiting branch (Oosterhuis and Jernstedt 1999). This results in the presence of bolls of various ages on a cotton plant.

Through each of the five weeks of flowering, the proportion of bolls from younger cohorts declined while there was a simultaneous increase in the proportion of bolls representing older cohorts. The dynamic nature in which a cotton plant produces bolls is associated with a decline in vegetative growth and cessation of flowering, which is referred to as “cut-out” (Guinn 1986). Physiological “cut-out” generally occurs at growth stage of five main stem nodes above a

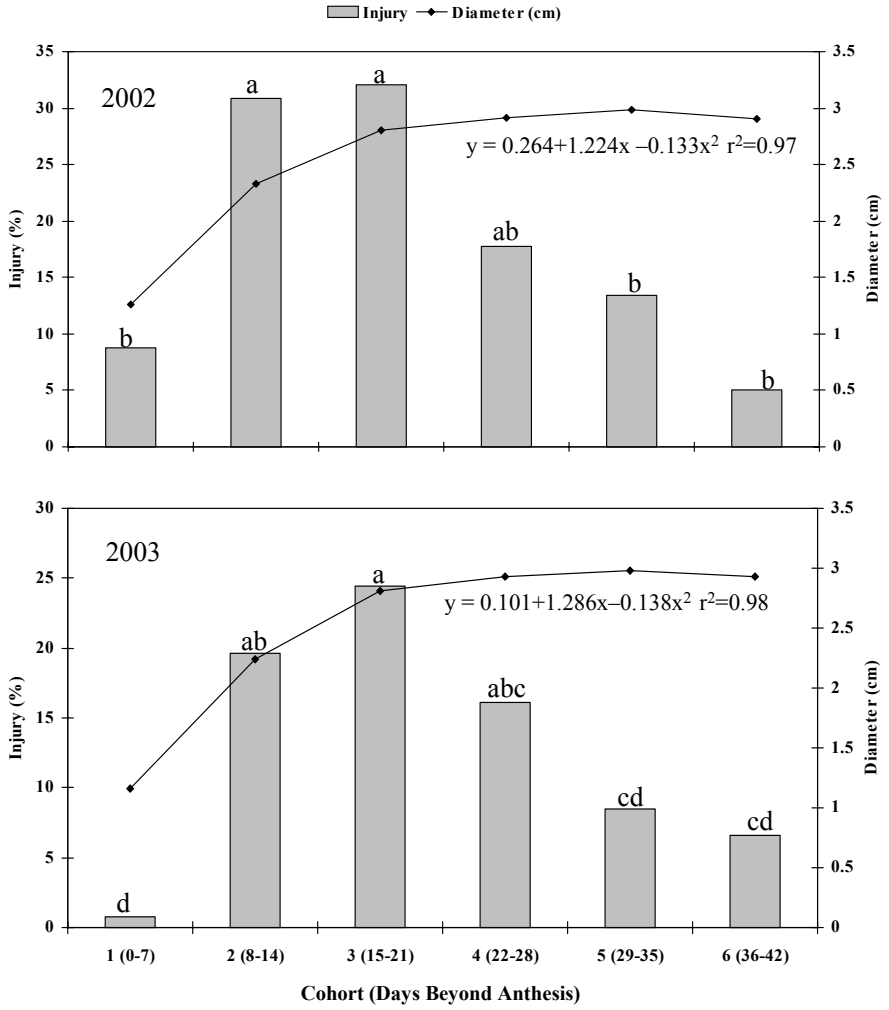

Figure 4.3. Relationship between boll cohort [days (based upon the accumulation of ca. 25 heat units per day)], diameter, and injury across the five weeks of flowering as influenced by brown stink bug infestations, 2002 and 2003. Injury in the infested treatment is corrected for injury within the non-infested treatment. Column means followed by the same letter are not significantly different ($P < 0.05$, LSD). The equation represents diameter as a function of cohort.

sympodial branch with a flower on the first node (Cothren 1999). Cotton in our studies reached "cut-out" by week four and five.

Brown stink bug injured bolls of different ages and in different proportions during the initial five weeks of flowering in this study. Generally, bolls from cohort two (165.3-330.5 heat units in 2002 and 169-336 heat units in 2003), cohort three (330.6-495.8 heat units in 2002 and 337-504 heat units in 2003), and cohort four (495.9-661.1 heat units in 2002 and 505-672 heat

units in 2003) were injured most by brown stink bug during the initial three weeks of flowering. When significant injury among boll cohorts was observed within a week, injury was lowest in the youngest bolls (cohort one, ≤168 heat units).

The relationship between boll density and boll injury also provides explanation for the preference by brown stink bug for particular boll cohorts. During the initial three weeks in 2002 and during week one in 2003, bolls from cohort one were present in greater densities compared to other cohorts. Percent injury was generally lower in boll cohort one compared to other cohorts due to a greater difference in the ratio of total bolls to injured bolls. The probability of locating a boll within this cohort would have been greater than for bolls of other cohorts. During 2003 in weeks two and three, the density of bolls in cohort one was similar to that of cohort two. However, boll injury was generally greater in cohort two compared to cohort one. Stink bugs distinctly preferred bolls from cohort two compared to cohort one because the ability of stink bugs to locate bolls should have been similar between the two cohorts. The influence of total fruit density on percent stink bug-injured fruit has been similarly observed in studies with tomato, *Lycopersicon esculentum* Miller (Zalom et al 1997). *Euschistus conspersus* Uhler and *Chlorochroa uhleri* (Stäl) adults were introduced onto caged, bush-type tomatoes possessing four fruit maturities: red, blush, large green (>2.5 cm diameter), and small green (<2.5 cm diameter). A negative relationship was observed between the percentage of fruit injured and number of fruit per plot when the stink bug density was held constant. Zalom et al. (1997) further suggested fruit density should be an important factor to consider when determining an action level for management of stink bugs in tomato, because low fruit density should increase percent fruit injury.

In both years during week four and during week five in 2003, brown stink bug did not demonstrate a preference for any boll cohort. Compared to previous weeks (one, two, and three), the plant canopy was much larger, boll cohorts were present on the plant in more equal proportions, and younger bolls were present on sympodial positions beyond the first position (boll cohort one). These conditions may have impaired the ability of brown stink bug to select specific bolls in the presence of a high density of other bolls. However, during week five in 2002, bolls in cohorts two and three (165.3-495.8 heat units) were most frequently injured by stink bugs. Boll density of these cohorts was greater than other cohorts, thus the opportunity for brown stink bug to locate these bolls was also greater. Brown stink bug preference for cohorts two and three is evident because in weeks one, two, and three, bolls in cohort one were present in large proportions but were not frequently injured.

Other studies have demonstrated reduced preference for smaller, younger fruit by stink bugs. In a cotton choice test, Lee et al. (1999) observed low levels of internal injury for bolls <3 d beyond anthesis. Southern green stink bug and green stink bug were caged on sympodial branches possessing a first (12-15 d beyond anthesis), second (6-9 d beyond anthesis), and third (0-3 d beyond anthesis) position boll. Internal injury was 3.1 and 3.8-fold greater in first and second position bolls, respectively, compared to third position bolls on the same sympodial branch. Similarly, Zalom et al. (1997) determined stink bugs feed on tomato fruit in any maturity categories, but prefer feeding on fruit larger than 2.5 cm in diameter.

The preference by brown stink bug for particular boll cohorts within a week was similar when cohorts were combined across weeks. Brown stink bug always caused significantly more injury to bolls in cohort two and three compared to cohorts one, five, and six. Boll injury observed in cohort four was intermediate compared to other cohorts. The proportion of bolls

injured in cohorts one, five, and six may have been lower than cohorts two and three because of their respective boll ages and diameters. However, the proportion of injury within cohorts two and three may have also been greater due to morphological and physiological characteristics of the boll that were attractive to stink bugs compared to other boll cohorts.

In cotton, boll development includes both a seed and fiber component. Boll size is largely influenced by fiber elongation. Fiber length increases rapidly in the first several days beyond anthesis, with the greatest increase in elongation at 12 d. Final length is attained at 27 d beyond anthesis (Schubert et al. 1973). Leffler (1976) has shown final boll size, as measured by fresh and dry weight, occurs at 21 to 28 d beyond anthesis. Beyond this point, the rate of fiber weight increase declines to zero at 55 d beyond anthesis (Schubert et al. 1973). Declining diameters in older bolls are related to losses in moisture associated with boll maturity (DeLanghe 1986). This pattern of boll growth and fiber elongation closely approximates that of boll diameter in our studies. These results are also similar to those reported in Chapter 2, in which there was a quadratic relationship between boll diameter and age. In those studies individual bolls of ages 0 through ca. 900 heat units beyond anthesis were infested with a single brown stink bug adults for 3 d or non-infested.

Seed development in cotton bolls occurs simultaneous to fiber elongation and has been described by Stewart (1986). Cell division and dry matter accumulation in the seed integument occurs during the initial 5 to 6 d and 16 to 17 d beyond anthesis, respectively. Internal seed weight initially increases after 4 to 6 d beyond anthesis, with greatest rates of increase occurring through 20 d beyond anthesis, the time at which protein accumulation begins. At 25 d beyond anthesis, the maximum length of the embryo is reached and oil accumulation begins. Seed coat hardening occurs at 45 to 50 d beyond anthesis.

Bolls in cohort one were the smallest and most immature as compared to other cohorts. Lack of seed development in these bolls may have influenced the level of injury observed. Many heteropterans (especially infraorder Pentamorpha) prefer the reproductive parts of plants (flowers, ovules, ovaries, and ripening seeds) and development of all life stages is significantly increased when developing or mature seed are present (Stein 1985, Stam et al. 1987, Schaefer and Panizzi 2000). In our studies, bolls in cohort one were ≤7 d beyond anthesis, which coincided with the enlargement of the seed integument. The absence of internal seed development may have decreased the nutritional value of these bolls, therefore, resulting in fewer injured bolls. Minimal injury in boll cohort one may have also been a result of boll abscission. Brown stink bug have been documented as inducing abscission in bolls that have accumulated >0 to 350 heat units beyond anthesis (Chapter 2). Therefore, even if bolls in cohort one were fed upon and injured, abscission was likely to occur. Other studies previously described would support the low levels of injury observed in our youngest boll cohort (Zalom et al. 1997, Lee et al. 1999).

Boll cohorts five and six also sustained less injury compared to boll cohorts two and three. These bolls were larger and the most mature, corresponding to ages of 29-42 d beyond anthesis. During this period, seed were accumulating oil but maximum boll size and fiber length had been attained. Fewer bolls in the cohorts may have not displayed obvious signs of injury because of the ability of stink bugs to penetrate a more mature boll. Studies with tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), have demonstrated that internal injury on carpel walls is limited to bolls ≤12 d old (ca. 250 heat units beyond anthesis). Tarnished plant bugs do not penetrate or cause yield loss in bolls >12 d old (Greene et al. 1999, Russell 1999, Horn et al. 1999). In our studies, injury occurred in boll cohorts five and six, demonstrating brown stink

bug are capable of penetrating bolls of this maturity. In pecan, *Carya illinoensis* (Wangenh) K. Koch, stink bugs cause kernel spot which is the result of feeding punctures made after "shell hardening" and after the kernel is formed (Stein 1985, Yates et al. 1991). However, stink bugs prefer thin-shelled cultivars (Stein 1983). Although stink bugs may have the ability to penetrate hardened fruit, injury may be most evident in fruit with minimal barrier (thin carpel walls) to the developing seed inside. The higher frequency of injured bolls in cohorts two and three as compared to cohorts five and six in the present study may be associated with a preference for those bolls.

Brown stink bug injured bolls in all cohorts. However, the proportion of injury observed in boll cohorts two, three, and four was generally greater compared to other cohorts. Some factor, or combination of factors, may be responsible for the attractiveness of these bolls to brown stink bug. Boll cohorts two, three, and four occurred at 8 through 28 d beyond anthesis. The period from 8 to 21 d beyond anthesis (cohort 2 and 3) corresponded to rapid increases in dry matter and protein accumulation in the seed, and maximum rates of boll expansion and fiber elongation. The period from 22 to 28 d beyond anthesis (cohort four) corresponded to increases in boll size, maximum embryo length, and initial stages of oil deposition in the seed. Preference by brown stink bug for boll cohorts two, three, and four may be related to chemical (primary or secondary metabolites) and/or physical (size and color) characteristics of those bolls. According to proposed host recognition theories, plant tissue may be acceptable for feeding because they lack compounds that inhibit feeding; whereas, plant tissue may be rejected because of the presence of deterrents (Schoonhoven et al. 1998). Additionally, some insects use differences in reflectance intensity among plant species, or among leaves or organs within a plant as a visual

selection criterion (Schoonhoven et al. 1998). Visual and olfactory stimuli, acting alone or in combination, may influence the frequency of injury observed among these cohorts.

Boll cohort did not significantly interact with the type of injury a particular boll displayed (single locule injury and multiple locule injury). The frequency of injured bolls within each cohort and during each week was similar, regardless of injury to one locule or multiple locules. These results strengthen the conclusion that the proportion of injured bolls within particular cohorts can be greater than in other cohorts during a week of flowering. However, multiple locule injury may not equate to a specific boll cohort being nutritionally acceptable to stink bugs. Multiple locules may be injured within bolls because more feeding events may be necessary to cause satiation. In contrast, an older boll with more developed seed may only require one feeding event to cause satiation. Lye and Story (1988) determined the frequency of feeding, as indicated by the presence of stylet sheaths was greater on green tomato fruit compared to red (fully ripened, mature) fruit. However, true feeding preference of southern green stink bug on tomato fruit could not be determined by the number of stylet sheaths alone. Other factors needing examination to determine true feeding preference include target tissue for feeding, the amount of liquid intake during each probing, and the influence of fruit color and chemical compounds in the fruit on the initiation and termination of feeding. Additionally, the presence of multiple injured locules in a boll could be due to feeding events by more than one stink bug. Concentration of stink bug adults within a field is not unusual and is related to the presence of aggregation pheromones (Todd and Herzog 1980).

Bolls that have accumulated 165.2 through 672 heat units beyond anthesis (ca. 7 to 27 d old) are more frequently injured by brown stink bug than bolls with other heat unit accumulations. However, this preference was less distinct during week four and five when bolls

were available in greater quantities and more equal proportions. Although injury was observed in boll cohorts accumulating through 672 heat units, previous studies demonstrated yield losses from brown stink only occur in bolls that have accumulated ≤550 heat units beyond anthesis (Chapter 2). The susceptible boll ages in our studies corresponded to a boll diameter of 1.161-3.586 cm with a mid-range of 2.375 cm. The most common boll ages and sizes injured by stink bugs, are broader than described for currently recommended sampling protocols [(12 to 16 d old boll and quarter-size (2.426 cm diameter)]. However, the specific boll size recommended for sampling occurs within this proposed range. Crop managers that sample bolls and determine the frequency of injured bolls in a cotton field as a means for initiating treatments against stink bugs should find this information useful. Sampling bolls within our defined boll age and size, which are likely to be injured, should make this method more reliable in detecting stink bug infestations.

References Cited

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 2002. Cotton insect, disease, nematode, and weed control recommendations for 2002. Alabama Cooperative Extension Service. http://www.aces.edu/dept/extcomm/ publications/anr/2002IPM/COTTON.02.PDF.

Bachelor, J.S. and J.W. Van Duyn. 2003. 2003 North Carolina agricultural chemical manual: insect control on cotton. http://ipm.ncsu.edu/agchem/ chptr5/505.pdf.

Bagwell, R.D. and N.P. Tugwell. 1992. Defining the period of boll susceptibility to insect damage in heat-units from flower, pp. 767-768. *In* D. Hebert and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bagwell, R.D., Stewart, S., B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2002. Cotton insect control, 2002. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Barbour, K.S., J.R. Bradley, and J.S. Bachelor. 1990. Reduction in yield and quality of cotton damaged by green stink bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 83: 842-845.

Boyd, M.L. and B.J. Phipps. 2003. University of Missouri cotton insect management guide-2003. http://www.aes.missouri.edu/delta/muguide/ctnmgtgs.stm#sbugs.

Bundy, C.S., R.M. McPherson, and G.A. Herzog. 2000. An examination of the external and internal signs of cotton boll damage by stink bugs (Heteroptera: Pentatomidae). J. Entomol. Sci. 35: 402-410**.**

Cothren, J.T. 1999. Physiology of the cotton plant, pp. 207-268. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

DeLanghe, E.A.L. 1986. Lint development, pp. 325-349. *In* J.R. Mauney and J.M. Stewart [eds.], Cotton physiology. The Cotton Foundation, Memphis, TN. 786 pp.

Greene, J.K. and G.A. Herzog. 1999. Management of stink bugs using symptoms of boll injury as a monitoring tool, pp. 1041-1044. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tanished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Guinn, G. 1986. Hormonal relations during reproduction, pp. 113-136. *In* J.R. Mauney and J.M. Stewart [eds.], Cotton physiology. The Cotton Foundation, Memphis, TN.

Horn, T.O., F.A Harris, J.T. Robbins, and R.E. Furr, Jr. 1999. Influence of boll age on susceptibility to tarnished plant bug injury, pp. 1044-1045. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Johnson, D.R., G.E. Studebaker, G. Lorenz, and J.K. Greene. 2002. Cotton insect management. University of Arkansas Cooperative Extension Service. http://www.uaex.edu./Other_Areas/publications/PDF/FSA-2065.PDF.

Lee, G.R., P. Roberts, and M.A. Abney. 1999. Stink bugs in cotton: feeding and injury observations, pp. 1036-1038. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leffler, H.R. 1976. Development of cotton fruit I: accumulation and distribution of dry matter. Agron. J. 68: 855-857.

Lye, B.H. and R.N. Story. 1988. Feeding preference of the southern green stink bug (Hemiptera: Pentatomidae) on tomato fruit. J. Econ. Entomol. 81: 522-526.

Oosterhuis, D.M. and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant, pp. 175-206. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY. 850 pp.

Patrick, C.R. and G.L. Lentz. 2001. 2001 Cotton insect control guide. University of Tennessee Agricultural Extension Service. Pub No. PB387-1M-1/01.

Roberts, P.M., D. Jones, and J. Ruberson. 2003. 2003 Georgia pest management handbook. http://www.ent.uga.edu/phh/Agronomic/AgronomicCrops.htm.

Russell, J.S. 1999. Effects of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feeding on cotton boll abscission and yield. M.S. thesis, Louisiana State University, Baton Rouge, LA. 47 pp.

SAS Institute. 1998. SAS User's manual, version 8. SAS Institute, Cary, NC.

Schaefer, C.W. and A.R. Panizzi. 2000. Economic importance of Heteroptera: a general overview, pp. 3-8. *In* C.W. Schaefer and A.R. Panizzi [eds.], Heteroptera of economic importance. CRC Press, Boca Raton, FL. 828 pp.

Schoonhoven, L.M., T. Jeremy, and J.J.A. van Loon. 1998. Insect-plant biology: from physiology to evolution. Chapman and Hall, London, UK. 409 pp.

Schubert, A.M., C.R. Benedict, J.D. Berlin, and R.J. Kohel. 1973. Cotton fiber elongation-kinetics of cell elongation and secondary wall thickening. Crop Sci. 13: 704-709.

Stam, P.A., L.D. Newsom, and E.N. Lambremont. 1987. Predation and food as factors affecting survival of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in a soybean ecosystem. Environ. Entomol. 16: 1211-1216.

Stein, L.A. 1985. Stink bugs: proper management can control kernel spots and black pit. Pecan South 19: 15-18.

Stewart, J.M. 1986. Integrated events in the flower and fruit, pp. 261-300. *In* J.R. Mauney and J.M. Stewart [eds.], Cotton physiology. The Cotton Foundation: Memphis, TN. 786 pp.

Todd, J.W. and D.C. Herzog. 1980. Sampling phytophagous pentatomidae on soybean, pp. 438-478. *In* M. Kogan and D.C. Herzog [eds.], Sampling methods in soybean entomology. Springer-Verlag, New York, NY.

Turnipseed, S.G., M.J. Sullivan, A.M. Hagerty, and A.L. Gibson. 2003. Boll damage by the stink bug/plant bug complex in South Carolina, pp. 1015. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Wene, G.P. and L.W. Sheets. 1964. Notes on control of stink bugs affecting cotton in Arizona. J. Econ. Entomol. 57: 60-62.

Willrich, M.M., D.R. Cook, and B.R. Leonard. 2003. Sampling hemipteran pests in cotton: a challenge to cotton IPM, pp. 73-64. Proceedings, Fourth National Integrated Pest Management Symposium. Indianapolis, IN.

Zalom, F.G, J.T. Smilanick, and L.E. Ehler. 1997. Fruit damage by stink bugs (Hemiptera: Pentatomidae) in bush-type tomatoes. J. Econ. Entomol. 90: 1300-1306.

Yates, I.E., W.L. Tedders, and D. Sparks. 1991. Diagnostic evidence of damage on pecan shells by stink bugs and coreid bugs. J. Amer. Soc. Hort. Sci. 116: 42-46.

CHAPTER 5

INFLUENCE OF SOUTHERN GREEN STINK BUG ON LATE-SEASON YIELD LOSSES IN COTTON

Introduction

Plant disease epidemics from boll (fruit) rotting pathogens contribute to reduced yield and quality in cotton, *Gossypium hirsutum* L., in most years. Boll rot ranks second to the seedling disease complex as the most destructive disease of cotton in the United States (Kirkpatrick and Rothrock 2001). In Louisiana, yield losses associated with boll rot are particularly severe. From 1991 to 2002 in Louisiana, losses ranged from 3.5 to 88.4 g lint per acre (National Cotton Council 2002). Boll rot is manifested as complete tissue decay by pathogens (Pinckard et al. 1981).

The climate in Louisiana can be very conducive to the development of boll rotting pathogens. Epidemics occur when excess moisture and humidity is present just prior to and during boll opening (August through September) (Roncadori et al. 1975, Padgett et al. 2003). Cultural practices and field environments that promote a dense foliage canopy also can result in a humid microclimate optimum for pathogen growth. Dense plant populations, excessive fertilizer rates, lack of using plant growth regulators, or reduced row widths will improve conditions for the occurrence of boll rot (Pinckard et al. 1981, Baker and McKinion 1995). Fungal and bacterial boll rotting pathogens can infect through wounds caused by insects, mechanical agents, and other plant pathogens, or intact bolls through natural openings (stomata and nectaries) (Kiyomoto and Ashworth 1974, Roncadori 1974, Kirkpatrick and Rothrock 2001).

Fungicide treatments are ineffective in preventing boll rot (Roncadori et al. 1975). Disease control measures have primarily been directed toward managing plant growth

that promotes increased sunlight penetration and reduces the humidity within the plant canopy (Snow et al. 1981). Boll rot has been reduced by 1.8-fold following a single, mid-season application of a commonly used plant growth regulator in cotton (N-N-Dimethylpiperidiniumchloride) (Snow et al. 1981). Effective control of insects that damage bolls also helps to reduce infection by some boll rotting pathogens (Kirkpatrick and Rothrock 2001).

Several of the factors associated with boll rot in cotton may also cause yield losses in the form of hard locked bolls. Hard lock is a condition in which individual carpels within a boll remain compact and fail to open normally (Pinckard et al. 1981, Marois et al. 2002). The etiology of the malady is unknown, however it has been associated with excess nitrogen, high temperature and humidity, high plant density, excess moisture at boll dehiscence (opening) insect injury, seed rot, and pathogens (Pinckard et al. 1981, Jones et al. 2000, Marois et al. 2002). Marois et al. (2002) inoculated *Fusarium* spp. and *Pestalotia* spp. into sympodial branches ca. 1 mo before harvest and increased the number of hard locked carpels (≤ 3.5-fold) per sympodial branch. Jones et al. (2000) observed a positive correlation between bolls with seed rot symptoms and the occurrence of hard locked bolls at boll opening. Barbour et al. (1990) determined the proportion of harvestable carpels per boll decreased as the duration of infestation and number of punctures per boll by green stink bug, *Acrosternum hilare* (Say), increased. Although hard locked bolls were not mentioned in this study, harvestable locks were defined as those whose lint was fluffy and could be collected by simulating mechanical harvesting.

Although the symptomology of hard locked and rotted bolls may differ, both

conditions reduce seedcotton yield. The reduction in yield due to boll rot is loss of entire bolls. In contrast, for hard locked bolls, mechanical pickers are impaired from harvesting seedcotton that exists as compacted, individual carpels within bolls (Marois et al. 2002).

Over the last decade, stink bugs have become more common in cotton and are likely to remain as significant pests (Greene et al. 1999, Leonard et al. 1999, Roberts 1999). Stink bugs feed on developing bolls and reduce lint yield and seed quality (Chapter 2, Wene and Sheets 1964, Barbour et al. 1990, Turnipseed et al. 1995, Greene et al. 1999). The contribution of these pests to boll rot and hard locked bolls will be critical in the development of management strategies. Direct control of boll rot with fungicides is inconsistent; therefore, management of the disease should be directed toward reducing factors that promote epidemics. Currently, no data is available that provide conclusive evidence that stink bugs increase the incidence of rotted and hard locked bolls when periods of high rainfall and humidity are present in cotton fields.

Materials and Methods

Studies were conducted at the Macon Ridge Research Station near Winnsboro, LA (Franklin Parish). The cotton cultivar 'DP458BR' was planted on 23 May and 30 Apr in 2002 and 2003, respectively, in plots of two rows (1.02 m centers) x 3.35 m. General agronomic and pest management practices recommended by the LSU AgCenter were used to maintain the test area. Plants were thinned to densities of nine plants per m within 3 wk after plant emergence. Translucent cages (32 nylon mesh/2.54 linear cm, Synthetic Industries, Greenville, Georgia) were placed over individual plots when plants attained a growth stage of ≤ 2 main stem nodes above a sympodial branch with a flower on the first node (NAWF) (22 Aug 2002 and 11 Aug 2003). This growth stage

corresponded to the sixth week of flowering. The first week of flowering was recorded when 50% of the plants across the study had ≥ 1 flower or boll.

Two treatments were arranged in a randomized complete block design with four replications. Treatments included caged plots infested with southern green stink bug, *Nezara viridula* (L.), (30 adults per cage) and caged non-infested plots. Southern green stink bugs were collected from soybean, *Glycine max* (L.) Merrill, ca. 24 h prior to infestation, using a standard 38.1 cm diameter sweep net. Stink bugs were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan) to reduce mortality from physical injury and were fed a small quantity of washed green beans, *Phaseolus vulgaris* (L.). In both years, stink bug infestations occurred on the same day cotton plots were caged during the sixth week of flowering.

The test area was irrigated to simulate persistent conditions of high rainfall and humidity beginning ca. 1 wk after stink bugs were infested on cotton plots. Natural and/or irrigated rainfall received by plots during 2002 and 2003 was 29.7 and 23.7 cm, respectively. Simulated rainfall (2.5 to 3.75 cm) was applied at ca. 3 to 4 d intervals if adequate natural rainfall did not occur. Infested cages were supplemented with 10 southern green stink bug adults for each of the four weeks after initial infestation. The cages were removed 5 wk after the initial date of infestation. Heat units were calculated for each day of infestation as: [(maximum daily temperature + minimum daily temperature)/2]-15.5, where 15.5°C (60°F) is the minimum adequate temperature for cotton plant development. In 2002, an average of 20.9 heat units per day were recorded for a total of 734.5 heat units during the study (35 d). In 2003, an average of 19.9 heat

units were accumulated per day for a total of 715.0 heat units (35 d). All non-target pests were suppressed with weekly insecticide applications at recommended rates throughout the growing season prior to infestation and after cages were removed from cotton plants.

All plots were chemically defoliated and all bolls were hand-harvested at a growth stage of ≤ 2 main stem nodes above a sympodial branch with a dehisced boll on the first node (NACB) in both years. Bolls were classified as rotted (tissue decay associated with pathogens), hard locked (at least one carpel [locule] in the boll with lint visible, but not open sufficiently to be harvested with a mechanical picker) or harvestable (normal, open bolls harvestable with a mechanical picker). The presence of stink bug injury was recorded for each hard locked and harvestable boll. Stink bug injury could not be detected within rotted bolls because of extensive tissue degradation. Injury was defined as the presence of lint discoloration with a corresponding wart (dried callous tissue) or puncture (dried circular tissue that previously had a water soaked appearance in green bolls) on the internal carpel wall (Bundy et al. 2000).

A cohort of rotted bolls was assayed to confirm the presence of boll-rotting pathogens. Excised tissue from the outer surface of bolls (external carpel walls) was surface sterilized with a 20% sodium hypochlorite solution for a period of 1.5 to 2 min. Each tissue sample was removed from the solution, blotted dry, and aseptically transferred to acidified potato dextrose agar (pH 4.5). Fungal pathogens were allowed to grow out of the tissue and classified to genus (Barnett and Hunter 1998). In another assay, carpels were removed from rotted bolls and rinsed in running water for ca. 5 min. External carpel walls were removed from bolls, placed on moist paper towels, and sealed inside plastic crisper boxes (35 cm x 20 cm x 10 cm) to induce sporulation of fungi

present on the carpel surface. Spores were used to classify pathogens to genus (Barnett and Hunter 1998).

Seedcotton collected from harvestable and hard locked bolls were separated into lint and seed with a laboratory gin. Weights for all yield components were measured. Seed germination was determined using the standard warm germination test for cotton seed (Association of Official Seed Analysts 2000). In 2002, an average of 562.5 and 829.5 seed per replication from harvestable and hard locked bolls, respectively, were randomly selected and germinated. In 2003, an average of 400 seed per replication from harvestable and hard locked bolls were germinated. The duration and temperature of incubation was 8 d and 30°C, respectively. The test measured the percentage of seedlings that have a combined hypocotyl and root length of 3.75 cm.

Analysis of variance (ANOVA) was used to test for significant interactions between year and treatment (stink bug-infested and non-infested) for each dependent variable ($\alpha = 0.05$) (PROC GLM, SAS Institute 1998). Percent rotted, hard locked, harvestable, stink bug-injured bolls, and seed germination was compared between the infested and non-infested treatments using ANOVA (SAS Institute 1998). Total seedcotton, lint, and seed yield data were also subjected to ANOVA, with boll density per plot used as a covariable in the model.

Results

Diplodia spp. and *Fusarium* spp. were isolated at the highest frequency from rotted bolls in both years. The incidence of *Diplodia* spp. in 2002 and 2003 was 88% and 98%, respectively. In 2002 and 2003, the incidence of *Fusarium* spp. was 52% and 2%, respectively.

The proportion of rotted, hard locked, and harvestable bolls were observed in equal proportions between treatments across 2002 and 2003. There was no significant interaction between year and treatment for percent rotted bolls ($F = 1.32$, df = 1,6, $P = 0.2945$), hard locked bolls ($F = 0.05$, df = 1,6, $P = 0.8232$), or harvestable bolls ($F = 0.01$, df = 1,6, $P = 0.9402$). Also, there was no significant interaction between year and treatment for percent stink bug injury in hard locked bolls ($F = 4.10$, df = 1,6, $P = 0.0673$) and in harvestable bolls ($F = 0.81$, df = 1,6, $P = 0.4026$).

Stink bugs significantly reduced the proportion of harvestable bolls in the infested treatment compared to the non-infested treatment (Table 5.1). The percentage of hard locked and rotted bolls within the stink bug-infested treatment was significantly greater than observed in the non-infested treatment. Stink bug injury was more common within hard locked and harvestable bolls (Table 5.1).

Table 5.1. Influence of southern green stink bug infestations on proportion of rotted, hard locked, and harvestable (normal) bolls, and presence of injury.

	% of Total Bolls ± SD			% Stink Bug-Injured ± SD[d]	
Treatment	Harvestable[a]	Hard Locked[b]	Rotted[c]	Harvestable Bolls	Hard Locked Bolls
Infested	77.7 ± 2.8b	18.0 ± 3.9a	4.3 ± 1.0a	20.3 ± 6.9a	35.8 ± 4.2a
Non-Infested	84.8 ± 4.4a	13.1 ± 4.2b	2.1 ± 0.8b	12.1 ± 4.9b	18.3 ± 10.4b
F	5.93	5.10	20.15	18.23	31.30
df	1,6	1,6	1,6	1,6	1,6
P>F	0.0407	0.0324	0.0042	0.0053	0.0014

Column means followed by the same letter are not significantly different ($P > 0.05$).
[a]Normal, open bolls harvested with a mechanical picker.
[b]≥ 1 carpel [locule] in the boll with lint visible, but not open sufficiently to be harvested with a mechanical picker.
[c]Tissue decay associated with pathogens.
[d]Presence of lint discoloration with a corresponding wart [dried callous tissue] or puncture [dried, circular spot that was formerly water-soaked in immature bolls] on the internal carpel wall.

There was no significant interaction between year and treatment for seedcotton harvested from hard locked ($F = 0.77$, df = 1,6, $P = 0.4218$) and harvestable ($F = 0.11$, df = 1,6, $P = 0.7529$) bolls. Also, there was no significant interaction between year and treatment for lint weight ($F = 0.01$, df = 1,6, $P = 0.9296$) and seed weight ($F = 0.15$, df = 1,6, $P = 0.7129$) of harvestable bolls. All components of yield data are combined for 2002 and 2003.

Stink bugs caused significantly more seedcotton to be produced from hard locked bolls in the infested treatment compared to the non-infested treatment (Table 5.2). In contrast, significantly greater yield was produced in harvestable bolls in the non-infested treatment compared to the stink-bug infested treatment. Stink bugs significantly reduced lint weight and total seed weight compared to the non-infested treatment.

Table 5.2. Influence of southern green stink bug infestations on seedcotton, lint, and seed yields in harvestable bolls and bolls exhibiting hard locked carpels.

	Yield / 22 row-ft (grams)			
	Hard Locked Bolls[a]	Harvestable Bolls[b]		
Treatment	Seedcotton	Seedcotton	Lint	Seed
Infested	250.6 ± 22.2a	1986.3 ± 63.6b	832.5 ± 24.4b	1129.6 ± 48.6b
Non-Infested	164.5 ± 22.2b	2175.2 ± 63.6a	916.6 ± 24.4a	1250.0 ± 48.6a
F	5.01	4.60	2.84	8.72
df	1,5	1,5	1,5	1,6
P>F	0.0377	0.0424	0.0413	0.0318

Column means followed by the same letter are not significantly different ($P > 0.05$).
[a]≥ 1 carpel [locule] in the boll with lint visible, but not open sufficiently to be harvested with a mechanical picker.
[b]Normal, open bolls harvested with a mechanical picker.

There was no significant interaction between year and treatment for germination of seed from hard locked ($F = 0.03$, df = 1,6, $P = 0.9977$) and harvestable ($F = 0.89$, df = 1,6, $P = 0.3828$) bolls (Table 5.3). Therefore, germination data will be presented across

2002 and 2003. No significant differences in germination were observed between the infested and non-infested treatment for seed collected from hard locked bolls. In contrast, stink bugs significantly reduced germination for seed collected from harvestable bolls compared to the non-infested treatment.

Table 5.3. Influence of southern green stink bug infestations on seed germination in hard locked and harvestable bolls.

	Seed Germination (%) ± SD			
Treatment	n[a]	Hard Locked Bolls[b]	n	Harvestable Bolls[c]
Infested	5,200	31.9 ± 4.0a	4,050	48.5 ± 9.6b
Non-Infested	4,636	33.3 ± 7.5a	3,650	57.1 ± 11.7a
F		0.07		4.77
df		1,6		1,6
P>F		0.7949		0.0358

Column means followed by the same letter are not significantly different ($P > 0.05$).
[a]Total number of seed germinated using the standard warm germination test for cotton seed.
[b]≥ 1 carpel [locule] in the boll with lint visible, but not open sufficiently to be harvested with a mechanical picker.
[c]Normal, open bolls harvested with a mechanical picker.

The year and boll classification (hard locked or harvestable) interaction for seed germination in the infested ($F = 3.78$, df = 1,6, $P = 0.0999$) and non-infested ($F = 5.69$, df = 1,6, $P = 0.0544$) treatments was not significantly different. Therefore, germination data were combined across years within each treatment to compare germination between hard locked and harvestable bolls. Germination was significantly less for seed collected from hard locked bolls compared to harvestable bolls in the infested ($F = 19.55$, df = 1,6, $P = 0.0045$) and non-infested ($F = 26.55$, df = 1,6, $P = 0.0021$) treatments.

Discussion

Diplodia spp. and *Fusarium* spp. were the most common boll-rotting fungi in our study. Both pathogens are documented as causal agents for boll rot in Louisiana and

across the southeastern United States (Sanders and Snow 1978, Kirkpatrick and Rothrock 2001). Sanders and Snow (1978) documented the conidia of *Alternaria gossypina*, *Curvularia spp*., *Helminthosporium gossypii, Diplodia gossypina*, and *Fusarium* spp. in airborne samples in Louisiana cotton fields from one week after flowers first appeared through harvest. *Diplodia* spp. and *Fusarium* spp. were collected in the greatest proportion in that study.

The initial density of adults per cage in our studies [8.2 adults/1.8 row-m (6 row-ft)] was 8.2-fold greater than the currently recommended level for initiating an insecticide treatment against stink bugs in cotton across several states [1 stink bug/1.8 row-m (6 row-ft)]. Infestation densities greater than recommended treatment thresholds were used to facilitate the interaction between inclement weather conditions and stink bugs. Minimal stink bug injury present in the non-infested treatment may have been caused by the immigration of other stink bugs or tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), into the study area between insecticide applications. Furthermore, tarnished plant bugs are capable of causing internal injury indistinguishable from that of stink bugs (Greene et al. 1999). Internal injury has been documented in bolls ≤ 12 d old (ca. 250 heat units beyond anthesis), but tarnished plant bugs do not penetrate or cause yield loss in bolls >12 d old (Greene et al. 1999, Russell 1999, Horn et al. 1999). Therefore, in our studies, the difference in boll injury observed between the infested and non-infested treatment should be associated with stink bugs infestations.

In the absence of stink bugs, high rainfall was capable of inducing rot and hard lock in cotton bolls. When stink bugs and high rainfall conditions occurred, rotted bolls and hard locked bolls were 2.0 and 1.4-fold greater, respectively, than observed in the

non-infested treatment. The presence of stink bugs, however, does not entirely justify the levels of hard locked and rotted bolls recorded in these studies. Although the level of stink bug injury in hard locked bolls was 1.9-fold greater in the infested treatment as compared to the non-infested, 64.2% of bolls did not have evidence of stink bug injury. Further, stink bug injury was observed on 20.3% of harvestable bolls. Future studies should investigate other factors possibly related to the development of hard locked bolls.

Southern green stink bug is capable of causing significant boll injury, yield losses, and reduced seed germination in cotton at growth stages beginning at ca. NAWF 2 (week six of flowering) plus 715-734.5 heat units. Previous studies have indicated stink bugs significantly reduce seedcotton weights of individual bolls that have accumulated ≤ 550 (ca. 22 d) heat units beyond anthesis (Chapter 2, Greene et al. 2001). In the present study, bolls of this age were present on cotton plants. Harvestable yield in the stink bug-infested treatment was reduced from complete lint decay (boll rot) and abnormal boll opening (hard lock). Developing bolls exposed to stink bugs and high rainfall will also result in reduced germination of seed collected from harvestable bolls. Germination of seed from harvestable bolls was 1.2-fold less in the stink bug infested treatment compared to the non-infested treatment. Therefore, harvestable seedcotton collected from the stink bug infested treatment sustained a loss in the form of reduced seed germination. Germination from hard locked bolls was not different between treatments; but, within each treatment, germination was lower in hard locked bolls compared to harvestable bolls. Once a boll becomes hard locked, then the presence of stink bugs does not further reduce seed germination.

Similar studies in soybean have demonstrated the incidence of seedborne

Fusarium spp. increased with increasing levels of stink bug [southern green stink bug; brown stink bug, *Euschistus servus* (Say); and green stink bug damage in seeds (Russin et al. 1988). Southern green stink bug adults have been documented as having the ability to transfer microorganisms during feeding (Ragsdale et al. 1979). Bacteria (31 species) and fungi (2 species), having originated within southern green stink bug, were isolated from seed in those studies. The authors concluded, however, that no more than a causal relationship exists between microbes and stink bugs.

The pathogens isolated from bolls in our studies are part of the normal microflora associated with boll rot. The primary effect of stink bug feeding may have been to modify the incidence of these microorganisms. Such effects may have been due to physical injury in bolls that resulted from stink bug feeding. Pinckard et al. (1981) suggested that insects can play a role in predisposing bolls to invasion by pathogens. Feeding may damage carpel walls and locules of bolls to the extent that boll opening is slowed and often imperfect (Pinckard et al. 1981). If carpels do not dehisce normally, lint does not dry rapidly and carpels are subject to microbial invasion. This condition would be especially pronounced during periods of persistent rainfall and humidity, as simulated in the present study.

Sanders and Snow (1978) have shown *Diplodia* spp. and *Fusarium* spp. are capable of rotting 40 d old [ca. 800 heat units beyond anthesis (based upon each day accumulating ca. 20 heat units)], mature bolls within 1 to 2 weeks after inoculation. Brown stink bug can injure bolls of any age, including older bolls accumulating 841 to 1014 heat units beyond anthesis (Chapter 3). However, brown stink bug prefer to injure bolls in cohorts that have accumulated 165 to 672 heat units beyond anthesis (Chapter 3).

In the present study, stink bugs had the opportunity to feed on bolls formed before infestations occurred and on younger bolls (≤ 715-735.5 heat units accumulated beyond anthesis).

Brown stink bug infestations occurring during week five of flowering (NAWF ≤ 3) can reduce seedcotton yield (Chapter 4). These results, combined with those of the present study, suggest stink bugs should be controlled during the commencing stages of flowering. Removal of infestations during week five of flowering may reduce yield losses, but also influence the levels of rotted and hard locked bolls if inclement weather (persistent rainfall and humidity) delay harvest.

References Cited

Association of Official Seed Analysts (AOSA). 2000. Rules for seed testing. AOSA, Las Cruces, NM.

Baker, D.N. and J.M. McKinion. 1995. Plant height and cotton boll rot, pp. 1101-*1103. In* D. Richter and J. Armour [eds.], Proc. 1995 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Barbour, K.S., J.R. Bradley, and J.S. Bachelor. 1990. Reduction in yield and quality of damaged by green stink bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 83: 842-845.

Barnett, H.L. and B.B. Hunter. 1998. Illustrated genera of imperfect fungi. American Phytopathological Society Press, St. Paul, MN. 218 pp.

Bundy, C.S., R.M. McPherson, and G.A. Herzog. 2000. An examination of the external and internal signs of cotton boll damage by stink bugs (Heteroptera: Pentatomidae). J. Entomol. Sci. 35: 402-410**.**

Greene, J.K., S.G. Turnipseed, M.J. Sullivan, and G.A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council. Memphis, TN.

Horn, T.O., F.A Harris, J.T. Robbins, and R.E. Furr, Jr. 1999. Influence of boll age on susceptibility to tarnished plant bug injury, pp. 1044-1045. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Jones, M.A., J.D. Mueller, D.A. Kluepfel, M.J. Sullivan, J.T. Walker, M.E. Roof, J. McD. Stewart, and D.E. Linveill. 2000. Preliminary investigations on cotton seed rot in South Carolina. South Carolina Agriculture and Forestry Research Station Bulletin 675. 19 pp.

Kirkpatrick, T.L. and C.S. Rothrock. 2001. Compendium of cotton diseases. Second Edition. American Phytopathological Society, St. Paul, MN. 74 pp.

Kiyomoto, R.K. and L.J. Ashworth. 1974. Status of cotton boll rot in the San Joaquin valley of California following simulated pink bollworm injury. Phytopathology 64: 259-260.

Leonard, B.R., J.B. Graves, and P.C. Ellsworth. 1999. Insect and mite pests of cotton. *In* C.W. Smith [ed.], Cotton: origin, history, technology, and production. John Wiley and Sons, Inc., New York, NY.

Marois, J.J., D.L. Wright, P.J. Wiatrak, and M.A. Vargas. 2002. Association of *Fusarium* spp. with hardlock of cotton in the southeastern US, CD-ROM C017.pdf. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

National Cotton Council. 2002. Cotton pest loss database, CD-ROM, loss data.pdf. National Cotton Council, Memphis, TN.

Padgett, G.B., D.J. Boquet, W. Rea, J.P. Caylor, and C. Shivers. 2003. Boll rot epidemics in Louisiana cotton, p. 211. *In* P. Dugger and D. Richter [eds.], Proc. 2003 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Pinckard, J.A., L.J. Ashworth, J.P. Snow, T.E. Russell, R.W. Roncadori, and G.L. Sciumbato. 1981. Boll rots, pp. 20-24. *In* G.M. Watkins [ed.], Compendium of cotton diseases. American Phytopathological Society, St. Paul, MN. 87 pp.

Ragsdale, D.W., A.D. Larson, and L.D. Newsom. 1979. Microorganism associated with feeding and from various organs of *Nezara viridula*. J. Econ. Entomol. 72: 725-727.

Roberts, P. 1999. Observations of emerging pests in low spray environments, p. 1034. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Roncadori, R.W. 1974. A new look at the cotton boll rot complex and promising approaches to control, pp. 155-158. *In* T. Nelson [ed.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roncadori, R.W., S.M. Carter, and J.L. Crawford. 1975. Evaluation of control measures for cotton boll rot. Phytopathology 65: 567-570.

Russell, J.S. 1999. Effects of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feeding on cotton boll abscission and yield. M.S. thesis, Louisiana State University, Baton Rouge. 47 pp.

Russin, J.S., D.B. Orr, M.B. Layton, and D.J. Boethel. 1988. Incidence of microorganisms in soybean seeds damaged by stink bug feeding. Phytopathology 78: 306-310.

Sanders, D.E. and J.P. Snow. 1978. Dispersal of airborne spores of boll-rotting fungi and the incidence of cotton boll rot. Phytopathology 68: 1438-1441.

SAS Institute. 1998. SAS User's manual, version 8. SAS Institute, Cary, NC.

Snow, J.P., S.H. Crawford, G.T. Berggren, and J.G. Marshall. 1981. Growth regulators tested for cotton boll rot control. La. Agric. 24: 3,24.

Turnipseed, S.G., M.J. Sullivan, J.E. Mann, and M.E. Roof. 1995. Secondary pests in transgenic Bt cotton in South Carolina, pp. 768-769. *In* D. Richter and J. Armour [eds.], Proc. 1995 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Wene, G.P. and L.W. Sheets. 1964. Notes on control of stink bugs affecting cotton in Arizona. J. Econ. Entomol. 57: 60-62.

CHAPTER 6

LABORATORY AND FIELD EVALUATIONS OF INSECTICIDE TOXICITY AGAINST STINK BUGS (HETEROPTERA: PENTATOMIDAE)*

Introduction

Insecticides are the primary tool used to manage stink bug infestations in cotton, *Gossypium hirsutum* L. Proper species identification and life stage characterization are necessary because differential insecticide susceptibility has been reported to vary among species and life stages (McPherson et al. 1979). McPherson et al. (1979) demonstrated morningglory stink bug, *Edessa bifida* (Say), adults had a significantly higher methyl parathion LD_{50} than adults of other stink bug species. The methyl parathion LD_{50}'s of southern green stink bug, *Nezara viridula* (L.), green stink bug, *Acrosternum hilare* (Say), and brown stink bug, *Euschistus servus* (Say), fifth instar nymphs also were higher than that for their corresponding adults. Insecticide efficacy trials in soybean, *Glycine max* (L.) Merrill, indicate brown stink bug is more difficult to control with products recommended for southern green stink bug (Fitzpatrick et al. 2001).

In 2001, Louisiana's insecticide recommendations for cotton IPM were refined to distinguish between brown stink bug (*Euschistus* spp.) and southern green stink bug/green stink bug (Bagwell et al. 2001). Only the organophosphates (acephate, dicrotophos, and methyl parathion) are recommended for control of *Euschistus* spp.; however, both organophosphates and pyrethroids are recommended for control of southern green stink bug and green stink bug. Several other states across the southeastern United States have similar recommendations for stink bug pest management in cotton (Patrick and Lentz 2001, Anonymous 2002, Bagwell et al. 2002, Johnson et al. 2002, Boyd and Phipps 2003, Bachelor and Van Duyn 2003, Roberts et al. 2003).

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The incidence of stink bug problems in cotton will likely increase as broad-spectrum insecticide applications decrease against key lepidopteran pests (Stewart et al. 2001). Therefore, defining the differences in insecticide susceptibility among species and life stages will become a critical issue for selecting an insecticide. Additionally, it is important to continually evaluate the field performance of insecticides for future insect control recommendations in cotton. This is particularly significant for *Euschistus* spp. Fewer insecticides are effective against these species compared to southern green stink bug and green stink bug.

Specific studies were designed to modify the adult vial test (AVT) for stink bugs and establish dose-mortality data for selected insecticides among species and life stages. Additionally, insecticide performance at field application rates was evaluated against brown stink bug adults using treated plant tissue.

Materials and Methods

Insect Collections

Brown stink bug, southern green stink bug, and green stink bug adults and nymphs were obtained early-season (May and June) from mustard, *Brassica* spp., and corn, *Zea mays* L., and late-season (August and September) from soybean in northeast Louisiana during 2000, 2001, 2002, and 2003 (Franklin Parish). Another brown colored stink bug species, *Euschistus quadrator* Rolston, were collected as adults from soybean in south Louisiana during 2002 (Livonia, LA, Pointe Coupee Parish). Insects were collected using a standard 38.1 cm diameter sweep net or hand-removed from plants. Collections were made ca. 24-h prior to field and laboratory studies. Insects were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, BugDorm, Megaview Science Education

Services CO. Ltd., Taichung, Taiwan) for 24 h and fed washed green beans, *Phaseolus vulgaris* (L.), and peanut, *Arachis hypogae* L., seeds. After the 24 h period, a cohort of stink bugs were selected that displayed normal behavior, without obvious signs of physical injury or parasitism (Todd 1989).

Laboratory Studies

Adult vial test (AVT) procedures similar to those described by Plapp et al. (1987), Plapp et al. (1990), and Snodgrass (1996) were used to evaluate the activity of organophosphate (dicrotophos and acephate) and pyrethroid (bifenthrin, λ-cyhalothrin, cyfluthrin, and cypermethrin) insecticides against stink bug adults and nymphs (fourth-fifth instars) during 2001, 2002, and 2003**.** Stock solutions of acephate (99.6% w/w, Valent USA Corporation, Walnut Creek, CA), dicrotophos (98% w/w, Chem Service, West Chester, PA), bifenthrin (99% w/w, Chem Service, West Chester, PA), cyfluthrin (94.9% w/w, Bayer Crop Protection, Kansas City, MO), cypermethrin (59% w/w *cis*, 39% w/w *trans*, Chem Service, West Chester, PA), and λ-cyhalothrin (98.7% w/w, Syngenta Crop Protection, Greensboro, NC) were developed by dissolving technical grade samples in acetone. Dilutions were made from each stock solution to yield the desired insecticide concentrations. Insecticide concentrations (six to ten/compound/test) of organophosphate and pyrethroid insecticides ranged from 0.05 to 7.5 μg/vial and 0.001 to 10.0 μg/vial, respectively. The interior surface of 20 ml glass scintillation vials was coated with an insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were then rotated on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment until used in bioassays.

Stink bugs were introduced into insecticide-treated or non-treated vials (one adult or nymph/vial). A minimum of 10 insects for each species and/or life stage were subjected to each dose within a bioassay. No food source was provided for insects during the AVT. Mortality was determined four h after exposure. The criterion for mortality was the inability of the insect to assume an upright posture within five s after being dislodged from the vial. Bioassays conducted within a three week period for a particular species and/or life stage were pooled for data analysis. Mortality for treated vials was corrected for natural mortality in the non-treated vials using Abbott's formula (Abbott 1925). Corrected mortality data was subjected to probit analysis using Polo PC (LeOra Software, Berkeley, CA), and LC_{50} and 95% confidence intervals were estimated. The LC_{50} values were considered significant if 95% confidence intervals did not overlap (Robertson and Preisler 1992).

Field Studies

Field trials were conducted during the summers of 2001 and 2003 at the LSU AgCenter's Macon Ridge Research Station (Franklin Parish). Plots were planted to the cotton cultivars 'Suregrow 747', 'Stoneville 4691B', 'Deltapine 458BR', 'Stoneville 4793R', and 'Fiber Max 989BR' in trial 2001-A, 2001-B, 2001-C, 2003-A and C, and 2003-B and D, respectively. Cotton was managed using agronomic practices and pest control strategies as recommended by the LSU AgCenter. Plots were four rows on centers of 101.6 cm and 15.2 m in length. Treatments were arranged in a randomized block design with four replications.

Insecticide treatments were applied to cotton plots at a growth stage of four to seven nodes above first position white flower (NAWF) and 10 to 14 nodes above the

mainstem cotyledon during 2001 and 2003, respectively. Treatments included the following: acephate (Orthene 90S [Soluble Powder], 90.0% ai wt/wt, Valent USA Corporation, Walnut Creek, CA), dicrotophos (Bidrin 8EC [Emulsifiable Concentrate], 82.0% ai wt/wt, Amvac Chemical Corporation, Newport Beach, CA), bifenthrin (Capture 2EC, 25.1% ai wt/wt, FMC Corporation, Philadelphia, PA), λ-cyhalothrin (Karate-Z 2.08CS [Capsulated Suspension], 22.8% ai wt/wt, Syngenta Crop Protection, Greensboro, NC), cypermethrin (Ammo 2.5EC, 30.6% ai wt/wt, FMC Corporation, Philadelphia, PA), *z*-cypermethrin (Mustang Max 0.8EC, 9.6% ai wt/wt, FMC Corporation, Philadelphia, PA), and cyfluthrin (Baythroid 2EC, 25% ai wt/wt, Bayer Crop Science, Research Triangle Park, NC).

In trials 2001-A and 2001-B, treatments were applied on 7 Aug and 14 Aug, respectively, with a high-clearance sprayer calibrated to deliver 56.1 L/ha (six gallons/acre [GPA]) through TX-8 hollow cone nozzles (two/row) at 276 kPa (40 psi). In trial 2001-C, treatments were applied on 22 Aug with a hand-held CO_2 sprayer calibrated to deliver 131.8 L/ha (14.1 GPA) through TeeJet 8002 flat fan nozzles (two/row) at 207 kPa (30 psi). In trials 2003-A, B, C, and D, treatments were applied on 3, 10, 18, and 25 Jun, respectively, with a tractor mounted sprayer calibrated to deliver 93.48 L/ha (10 GPA) through TeeJet AI1100015VS flat fan nozzles (two/row) at 207 kPa (30 psi). At two to three h after application, 10 bolls (uppermost, first position quarter-size, one/plant) and 10 to 15 leaves (first fully expanded leaf below the last fully expanded terminal leaf) were collected per plot, during 2001 and 2003, respectively. No rainfall occurred between insecticide application and removal of plant tissue in each of the trials. Each boll was placed in a 0.09 L (three oz) plastic specimen vial and each leaf was placed in a

petri dish (100 x 15 mm) supplied with a moistened disk (8.9 cm) of filter paper. Vials and petri dishes were transported to the laboratory and infested with one brown stink bug. Specimen vials and petri dishes were stored in the laboratory under ambient conditions (ca. 26.7°C). Percent mortality was determined at 48 h after infestation (HAI). The criterion for mortality was the inability of the insect to assume an upright posture within five s after being dislodged from the vial. Data were analyzed with ANOVA and treatments were compared to the control in each trial using a Dunnet's one-tailed test (PROC GLM, SAS Institute 1998).

Results and Discussion

Laboratory (AVT) Studies

The LC_{50}'s for brown stink bug adults exposed to organophosphate insecticides ranged from 0.17 to 1.26 μg/vial (Table 6.1). Acephate was significantly more toxic (6.4

Table 6.1. Response of stink bug adults to insecticides at 4 h after exposure in the AVT, 2000-2002.

		Brown Stink Bug			Southern Green Stink Bug		
Insecticide	Year	n[a]	Slope ± SE	LC_{50} (95% CL)[b,c]	n[a]	Slope ± SE	LC_{50} (95% CL)[b,c]
Acephate	2001	210	3.14 ± 0.37	0.17 (0.12-0.26)	-----	-----	-----
Dicrotophos	2001	495	1.35 ± 0.14	1.09 (0.61-2.54)	-----	-----	-----
	2002	270	2.00 ± 0.22	1.26 (0.82-1.98)	270	1.85 ± 0.21	0.63 (0.40-0.94)
Bifenthrin	2000	330	2.65 ± 0.32	0.47 (0.38-0.59)	320	1.18 ± 0.15	0.58 (0.31-1.18)
	2001	675	1.55 ± 0.11	0.39 (0.33-0.46)	296	1.76 ± 0.18	0.24 (0.14-0.39)
	2002	240	1.03 ± 0.15	0.27 (0.18-0.43)	240	3.40 ± 0.58	0.10 (0.07-0.14)
Cyfluthrin	2000	280	1.92 ± 0.22	0.39 (0.26-0.55)	340	2.11 ± 0.24	0.10 (0.09-0.12)
Cypermethrin	2000	245	3.37 ± 0.42	0.92 (0.80-1.06)	400	2.52 ± 0.35	0.32 (0.21-0.42)
	2001	270	1.95 ± 0.22	0.87 (0.68-1.10)	-----	-----	-----
	2002	300	2.47 ± 0.24	1.69 (1.24-2.31)	390	1.33 ± 0.12	0.05 (0.03-0.08)
λ-cyhalothrin[d]	2000	335	1.97 ± 0.19	0.84 (0.71-0.99)	276	1.62 ± 0.21	0.11 (0.09-0.14)
	2001	559	1.27 ± 0.15	2.55 (1.39-9.33)	251	0.96 ± 0.19	0.05 (0.02-0.08)
	2002	303	1.44 ± 0.15	1.33 (0.56-4.52)	265	1.78 ± 0.30	0.02 (0.003-0.03)

Table 6.1. Continued.

[a]Number tested including controls.
[b]Concentrations reported in μg insecticide per vial.
[c]LC_{50} values significantly different if 95% confidence limits did not overlap.
[d]Response of *E. quadrator* adults to λ-cyhalothrin [Year: 2002; n = 222; Slope ± SE: 2.56 ± 0.37; LC_{50} (95% CL): 0.89 (0.67-1.14).

and 7.4-fold) to brown stink bug adults than dicrotophos. There was no significant difference between the responses of southern green stink bug adults and brown stink bug adults to dicrotophos.

The LC_{50}'s for brown stink bug adults exposed to pyrethroid insecticides ranged from 0.27 to 2.55 μg/vial (Table 6.1). Brown stink bug adults were most sensitive to bifenthrin and cyfluthrin, and least sensitive to λ-cyhalothrin and cypermethrin. Toxicity of λ-cyhalothrin to brown stink bug adults and *E. quadrator* adults was not significantly different in 2002.

The LC_{50}'s for southern green stink bug adults exposed to pyrethroid insecticides ranged from 0.02 to 0.58 μg/vial. The pyrethroid, λ-cyhalothrin (5.3, 4.8, and 5.0-fold), was significantly more toxic than bifenthrin to southern green stink bug adults during each year. Cypermethrin toxicity to southern green stink bug adults was similar to bifenthrin and λ-cyhalothrin in 2000 and 2002, respectively.

Toxicity of bifenthrin was generally similar between brown stink bug and southern green stink bug adults; however, in 2002, brown stink bug adults were significantly less sensitive (2.7-fold) than southern green stink bug adults. Similarly, *E. quadrator* adults were 8.1, 17.8, and 44.5-fold less sensitive to λ-cyhalothrin as compared to southern green stink bug adults. Southern green stink bug adults were

significantly more sensitive than brown stink bug adults to cyfluthrin (3.9-fold), cypermethrin (2.9 to 33.8-fold), and λ-cyhalothrin (7.6 to 66.5-fold).

The LC_{50}'s for brown stink bug and southern green stink bug nymphs exposed to pyrethroid insecticides ranged from 0.06 to 0.29 and 0.18 to 0.40 μg/vial, respectively (Table 6.2). Brown stink bug nymphs were significantly more sensitive to λ-cyhalothrin than bifenthrin. There were no significant differences among all pyrethroids in the responses of southern green stink bug nymphs. The response of brown stink bug, green stink bug, and southern green stink bug nymphs to λ-cyhalothrin was similar during 2002. The response of brown stink bug and southern green stink bug nymphs to bifenthrin was equal.

Significant differences were observed between adults and nymphs within a species in their responses to insecticides in the AVT (Tables 6.1, 6.2). Brown stink bug nymphs were significantly more sensitive (22.2-fold) to λ-cyhalothrin compared to brown stink bug adults. Southern green stink bug nymphs were less sensitive than southern green stink bug adults to cypermethrin and λ-cyhalothrin. Bifenthrin was equally toxic to brown stink bug and southern green stink bug, regardless of life stage.

Table 6.2. Response of stink bug nymphs to insecticides at 4 h after exposure in the AVT, 2001-2003.

		Brown Stink Bug			Southern Green Stink Bug		
Insecticide	Year	n[a]	Slope ± SE	LC_{50} (95% CL)[b,c]	n[a]	Slope ± SE	LC_{50} (95% CL)[b,c]
Bifenthrin	2002	-----	-----	-----	240	0.96 ± 0.14	0.18 (0.08-0.34)
	2003	230	1.62 ± 0.17	0.29 (0.18-0.47)	-----	-----	-----
Cypermethrin	2002	-----	-----	-----	390	1.41 ± 0.12	0.19 (0.12-0.28)
λ-cyhalothrin[d]	2001	-----	-----	-----	360	0.73 ± 0.13	0.40 (0.26-0.68)
	2002	200	0.71 ± 0.12	0.06 (0.02-0.14)	144	0.50 ± 0.16	0.22 (0.04-0.68)

[a]Number tested including controls.
[b]Concentrations reported in μg insecticide per vial.

Table 6.2. Continued.

[c]LC_{50} values significantly different if 95% confidence limits did not overlap.
[d]Response of green stink bug nymphs to λ-cyhalothrin [Year: 2002; n = 201; Slope ± SE: 0.89± 0.16; LC_{50} (95% CL): 0.08 (0.03- 0.14).

Similar results have been obtained in Mississippi using the AVT (G.L. Snodgrass, personal communication). Brown stink bug adults were observed to be more tolerant to organophosphate and pyrethroid insecticides compared to green stink bug adults and southern green stink bug adults. The LC_{50}'s for brown stink bugs exposed to organophosphate and pyrethroid insecticides were 1.0 to 11.6 and 0.9 to 13.9-fold greater, respectively, than LC_{50}'s for southern green stink bugs and green stink bugs.

Field Studies

In trials 2001-A, B, and C, mortality (67.5 to 85.0%) of brown stink bug adults exposed to bolls treated with acephate or dicrotophos was significantly greater than that on non-treated bolls ($P < 0.01$) (Table 6.3). Bolls treated with acephate [0.56 kg/ha (0.5 lb AI/acre)] resulted in mortality of brown stink bug adults that also was significantly greater than mortality on non-treated bolls ($P < 0.05$). Mortality of brown stink bug exposed to bifenthrin was significantly greater than that on non-treated bolls ($P < 0.05$) and was similar to that of acephate and dicrotophos. Bolls treated with λ-cyhalothrin did not produce mortality of brown stink bug that was significantly different from that on non-treated bolls ($P > 0.05$).

In trial 2003-A, there was a positive relationship between the rate of λ-cyhalothrin applied to leaf tissue and brown stink bug mortality (Table 6.3). Foliage treated with λ-cyhalothrin [0.034 kg/ha (0.03 lb AI/acre)] produced mortality of brown stink bug that was significantly greater than on non-treated foliage ($P < 0.01$). *Lambda*-cyhalothrin-

Table 6.3. Evaluation of insecticides for control of brown stink bug adults on cotton bolls and foliage at 48 h after infestation.

Test	Treatment	Rate/ha [kg AI (lb AI)]	Percent Mortality	*P* > *F* (ANOVA)
2001-Bolls				
Trial A	acephate	0.84 (0.75)	77.0**	0.0007
	bifenthrin	0.056 (0.05)	74.7**	
	λ-cyhalothrin	0.034 (0.03)	43.3	
	non-treated	-----	7.5	
Trial B	dicrotophos	0.28 (0.25)	67.5**	0.0001
	dicrotophos	0.45 (0.4)	85.0**	
	non-treated	-----	15.0	
Trial C	acephate	0.56 (0.5)	53.3*	0.0029
	acephate	1.12 (1.0)	73.3**	
	dicrotophos	0.45 (0.4)	76.7**	
	λ-cyhalothrin	0.028 (0.025)	23.3	
	non-treated	-----	6.7	
2003-Foliage				
Trial A	λ-cyhalothrin	0.011 (0.01)	5.9	0.0001
	λ-cyhalothrin	0.023 (0.02)	14.2	
	λ-cyhalothrin	0.034 (0.03)	30.8*	
	λ-cyhalothrin	0.045 (0.04)	76.7**	
	non-treated	-----	1.7	
Trial B	bifenthrin	0.057 (0.05)	56.7**	0.0003
	z-cypermethrin	0.028 (0.025)	61.5**	
	dicrotophos	0.56 (0.5)	78.3**	
	non-treated	-----	17.5	
Trial C	acephate	0.85 (0.75)	74.2**	0.0008
	bifenthrin	0.068 (0.06)	65.0**	
	cypermethrin	0.11 (0.1)	71.7**	
	non-treated	-----	6.7	
Trial D	cyfluthrin	0.045 (0.04)	52.5**	0.0001
	acephate	0.85 (0.75)	89.2**	
	non-treated	-----	0.0	

Significance based on Dunnett's one-tailed test (*$P < 0.05$, **$P < 0.01$).

treated foliage [0.045 kg/ha (0.04 lb AI/acre] also resulted in mortality of brown stink bug that was significantly greater than on non-treated foliage ($P < 0.05$). Lower rates of λ-cyhalothrin did not result in mortality significantly different from that on non-treated foliage ($P > 0.05$). In trials 2003-B, C, and D, acephate, bifenthrin, cyfluthrin, cypermethrin, *z*-cypermethrin, and dicrotophos produced mortality of brown stink bug significantly greater than on non-treated foliage ($P < 0.01$).

The organophosphate insecticides, acephate and dicrotophos are currently recommended for control of brown stink bug in Louisiana. These insecticides provided 48 h mortality on bolls and foliage ranging from 53.3 to 89.2%. *Lambda*-cyhalothrin provided mortality of brown stink bug adults comparable to the recommended insecticides (76.7%) at the highest labeled rate [0.045 kg AI/ha (0.04 lb AI/acre)]. High rates of other pyrethroids (bifenthrin, cypermethrin, *z*-cypermethrin, and cyfluthrin) demonstrated mortality (52.5 to 74.7%) of brown stink bug adults comparable to that of acephate and dicrotophos.

Field trials in cotton and soybean have provided evidence that organophophate insecticides, including acephate, dicrotophos, and methyl parathion, provide consistent and satisfactory control of green stink bug, southern green stink bug, and brown stink bug (McPherson et al. 1999a, McPherson et al. 1999b, Willrich et al. 2000, Fitzpatrick et al. 2001). Greene et al. (2001) demonstrated with topical application techniques that acephate [0.56 kg/ha (0.5 lb AI/acre)] and dicrotophos [0.28 and 0.56 kg/ha (0.25 and 0.5 lb AI/acre)] provided 69 to 100% mortality of adult and fifth instar nymphs of southern green stink bug and brown stink bug. Although McPherson et al. (1979) determined the laboratory response (LC_{50}) of stink bug nymphs (brown stink bug, green stink bug, and

southern green stink bug) was greater than their corresponding adults to methyl parathion, no field control failures with recommended organophosphate insecticides have been reported in the United States.

Results from field studies comparing the efficacy of insecticides between southern green stink bug adults and nymphs support results from the AVT in the present research. Southern green stink bug nymphs exposed to bolls treated with λ-cyhalothrin [0.034 kg/ha (0.03 lb AI/acre)] within 4 h after application resulted in 65% mortality at 24 h after exposure (Willrich et al. 2003). Southern green stink bug adults exposed to bolls at 24 h after treatment resulted in 97.5% mortality at 24 h after exposure. Based on these studies, southern green stink bug adults are highly sensitive to insecticides, particularly λ-cyhalothrin.

These data and that in other reports suggest that pyrethroids as a class are not equally toxic to all stink bug species. Topical applications of pyrethroid insecticides (bifenthrin, cypermethrin, *z*-cypermethrin, and cyfluthrin) resulted in 77 to 98% mortality of southern green stink bug adults and nymphs (Greene et al. 2001). In contrast, mortality of brown stink bug adults and nymphs ranged from 20 to 65%. Of the pyrethroids tested, bifenthrin, provided 65 and 63% mortality of brown stink bug adults and nymphs, respectively (Greene et al. 2001). Emfinger et al. (2001) demonstrated bifenthrin, applied at 0.056 and 0.078 kg/ha (0.05 and 0.07 lb AI/acre), controlled brown stink bug adults comparable to southern green stink bug adults when caged on cotton bolls. These results combined with the present data demonstrate bifenthrin to be active against several stink bug species and different life stages. Additionally, the 2003 field trials indicate high, labeled rates of other pyrethroids also may provide satisfactory

control of brown stink bug adults.

Based on these results, Alabama, Arkansas, Georgia, Louisiana, Missouri, North Carolina, and Tennessee are justified in differentiating insecticide recommendations between brown stink bug species and southern green stink bug/green stink bug in cotton (Patrick and Lentz 2001, Anonymous 2002, Bagwell et al. 2002, Johnson et al. 2002, Boyd and Phipps 2003, Bachelor and Van Duyn 2003, Roberts et al. 2003). In the future, initiating control measures against stink bugs in cotton will require more than detection of the stink bug pest complex and determination of the infestation level. Proper identification of species and developmental stages will be necessary because insecticide susceptibility varies among species and life stages.

Numerous products are recommended for management of southern green stink bug and green stink bug. However, insecticides representing the organophosphate class have provided the most consistent control of brown stink bugs. Tolerance re-assessment and re-registration is currently in progress for organophosphates, as directed under the Food Quality Protection Act of 1996. As additional restrictions for organophosphate use are implemented, it will be critical to evaluate the toxicity of registered and experimental insecticides against the complete spectrum of stink bug species found in cotton fields.

References Cited

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 2002. Cotton insect, disease, nematode, and weed control recommendations for 2002. Alabama Cooperative Extension Service. http://www.aces.edu/dept/extcomm/publications/anr/2002IPM/COTTON.02.PDF.

Bachelor, J.S. and J.W. Van Duyn. 2003. 2003 North Carolina agricultural chemical manual: insect control on cotton. http://ipm.ncsu.edu/agchem/ chptr5/505.pdf.

Bagwell, R.D., J.W. Barnett, B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2001. Cotton Insect Control, 2001. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Bagwell, R.D., Stewart, S., B.R. Leonard, G. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2002. Cotton Insect Control, 2002. Louisiana Cooperative Extension Service. Pub. No. 1083. 6 pp.

Boyd, M.L. and B.J. Phipps. 2003. University of Missouri cotton insect management guide-2003. http://www.aes.missouri.edu/delta/muguide/ctnmgtgs.stm#sbugs.

Emfinger, K., B.R. Leonard, J. Gore, and D. Cook. 2001. Insecticide toxicity to southern green, *Nezara viridula* (L.), and brown, *Euschistus servus* (Say), stink bugs, pp. 1159-1161. *In* P. Dugger and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Councilc, Memphis, TN.

Fitzpatrick, B.J., M.M. Willrich, D.J. Boethel, and B.R. Leonard. 2001. Evaluation of insecticides against stink bug on soybean, 2000. Arthropod Management Tests Vol. 26 F96. http://www.entsoc.org/Protected/AMT/AMT26/INDEX.ASP.

Greene, J.K., G.A. Herzog, and P.M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. *In* P. Dugger and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Johnson, D.R., G.E. Studebaker, G. Lorenz, and J.K. Greene. 2002. Cotton insect management. University of Arkansas Cooperative Extension Service. http://www.uaex.edu/Other_Areas/publications/PDF/FSA-2065.PDF.

McPherson, R.M., J.B. Graves, and T.A. Allain. 1979. Dosage-mortality responses and field control of seven pentatomids associated with soybean, exposed to methyl parathion. Environ. Entomol. 8: 1041-1043.

McPherson, R.M., M.L. Wells, and C.S. Bundy. 1999a. Control of stink bugs and velvetbean caterpillars on Georgia soybeans, 1998. Arthropod Management Tests Vol. 24 F110. http://www.entsoc.org/Protected/AMT/AMT24/ INDEX.ASP.

McPherson, R.M., J.D. Taylor, and B.D. Crowe. 1999b. Control of insect pests on soybeans, 1998. Arthropod Management Tests Vol. 24 F111. http://www.entsoc. org/Protected/AMT/AMT24/INDEX.ASP. (9 Nov 2003)

Patrick, C.R. and G.L. Lentz. 2001. 2001 Cotton insect control guide. University of Tennessee Agricultural Extension Service. Pub No. PB387-1M-1/01.

Plapp, F. W., G. M. McWhorter, and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas-1986, pp. 324-326. *In* T. Nelson [ed.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Plapp, F.W., J.A. Jackman, C. Campanhola, R.E. Frisbie, J.B. Graves, R.G. Luttrell, W.F. Kitten, and M. Wall. 1990. Monitoring and management of pyrethroid resistance in tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. J. Econ. Entomol. 83: 335-341.

Robertson, J.L. and H.K. Preisler. 1992. Pesticide bioassays with arthropods. CRC Press, Boca Raton, FL.

Roberts, P.M., D. Jones, and J. Ruberson. 2003. 2003 Georgia pest management handbook. http://www.ent.uga.edu/pmh/Agronomic/Agronomic_Crops.htm.

SAS Institute. 1998. SAS User's manual, version 8. SAS Institute, Cary, NC.

Snodgrass, G.L. 1996. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). J. Econ. Entomol. 89: 1053-1059.

Stewart, S.D., J.J. Adamczyk, K.S. Knighten, and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis*: baseline on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Todd, J.W. 1989. Ecology and behavior of *Nezara viridula*. Annu. Rev. Entomol. 34: 273-292.

Willrich, M.M., C.G. Clemens, M.E. Baur, B.J. Fitzpatrick, and D.J. Boethel. 2000. Evaluation of insecticides for southern green and brown stink bug control on soybean, 1999. Arthropod Management Tests Vol. 25 F152. http://www.entsoc.org/Protected/AMT/AMT25/INDEX.ASP.

Willrich, M.M., J. Temple, R.H. Gable, and B.R. Leonard. 2003. Evaluation of insecticides for control of southern green stink bug nymphs and residual efficacy against adults, 2002. Arthropod Management Tests Vol 28 F76. http://www.entsoc.org/Protected/AMT/AMT28/INDEX.ASP.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Cotton, *Gossypium hirsutum* L., insect pest management strategies are constantly evolving with changes in crop production practices, emergence of new pests, and development of novel pest control technologies. During the previous decade, producer participation in area-wide boll weevil, *Anthonomous grandis grandis* Boheman, eradication programs, development of target-specific insecticides, and introduction of Bollgard (*Bacillus thuringiensis*) cotton have caused entomologists to refine cotton insect pest management recommendations. Such practices have resulted in control of insecticide-resistant pest species, a general reduction in the use of broad-spectrum insecticides, and an increased abundance of hemipteran pests (stink bugs and plant bugs). The prevalence of stink bugs and plant bugs in cotton at the present time may also be related to an agricultural landscape that provides suitable hosts year-round. Wide-spread adoption of conservation tillage, crop rotation, and transfer of crop land into Conservation/Wetland Reserve Programs provide a broad range of host plants for hemipteran pests that subsequently infest cotton.

Across the mid-southern and southeastern United States, stink bugs typically occur as a complex of several species. The complex is comprised primarily of brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say). Historically, among row crops in these production regions, stink bugs have been most commonly associated with soybean, *Glycine max* (L.) Merrill, and corn, *Zea mays* L. Documentation of stink bug injury to cotton has largely been related to boll injury caused by southern green stink bug and

green stink bug. Brown stink bug have become more common in Louisiana and are historically less susceptible to insecticides compared to other stink bug species.

The impact of brown stink bug and southern green stink bug injury was evaluated on pre-flowering and flowering cotton plants in forced-feeding tests. Vegetative stage cotton seedlings and reproductive structures, including flower buds (square) and bolls, were infested with adults and/or nymphs of both species. There were no significant differences in height, height to node ratio, square retention, and flower initiation for cotton seedlings or plants with a match-head square between stink bug adult-infested and non-infested treatments. Abscission rates for individual large squares (pre-candle) and multiple squares (medium and small square on the same sympodial branch) were not significantly different ($P > 0.05$) among infested and non-infested treatments for brown stink bug adults, southern green stink bug adults, and third and fourth-fifth instar southern green stink bug nymphs.

In boll infestation studies, the relationship between boll maturity, expressed as heat units beyond anthesis, and boll growth (changes in diameter), boll abscission, hard locked carpels, seedcotton yield, and seed germination was measured. Brown stink bug induced abscission in bolls that accumulated > 0 to 350 heat units beyond anthesis. Boll growth and seedcotton yield was significantly lower for bolls infested with brown stink bug through 266.5 and through 550 heat units beyond anthesis, respectively, compared to non-infested bolls. The proportion of hard locked carpels per boll was significantly greater for the infested treatment in a cohort of bolls that accumulated from 51 to 400 heat units beyond anthesis. Seed germination in bolls infested with brown stink bug was

significantly lower in bolls aged 101 to 600 heat units beyond anthesis compared to that in non-infested bolls.

Results from forced-feeding tests indicated pre-flowering cotton was not significantly impacted by brown stink bug adults and southern green stink bug adults and nymphs. Bolls, however, were significantly injured by brown stink bug. Therefore, sampling for stink bugs and initiating treatment against them in cotton should be intensified during phenological stages of cotton growth corresponding to boll development. Brown stink bug can injure cotton bolls similar to that previously documented for southern green stink bug and green stink bug. Bolls that have accumulated ≥ 551 heat units beyond anthesis are tolerant to stink bug feeding and did not result in reduced seedcotton yield. This information can be incorporated into insecticide termination rules for cotton, which state that the last harvestable bolls for a cotton crop should be protected from a particular insect pest until economic injury by the pest can be avoided.

In general, sampling and initiating treatments against stink bugs is difficult due to their mobility, in-field distribution, and host range. For cotton, the problem is more complex because dense, tall canopies make sampling with sweep nets and shake sheets a cumbersome task for estimating stink bug densities. Therefore, a sampling protocol that estimates boll injury rather than stink bug density for a cotton fields is being developed across the mid-southern and southeastern United States. In Louisiana, the effects of brown stink bug infestations during each of the initial five weeks of flowering was studied to define cotton boll cohorts most frequently injured during each week and across weeks. Bolls ranging in age from 22.5 to 1035.5 heat units beyond anthesis were

grouped into one of six cohorts. The interval of each cohort was based upon the average number of heat units accumulated during a seven day period. Additionally, the relationship between boll injury within a week to losses in yield was evaluated.

Generally, brown stink bug injured significantly more bolls from cohort two (ca. 165-336 heat units), cohort three (ca. 330-504 heat units), and cohort four (ca. 495-672 heat units) during the initial three weeks in both years and in week five in 2002. The frequency of injured bolls was lowest in cohort 1 (≤ 168 heat units) during these same weeks. In both years during week four and during week five in 2003, the proportion of injured bolls among cohorts was similar. The preference by brown stink bug for boll cohorts 2, 3, and 4 within a week was similar when cohorts were combined across all five weeks. Based on these data, bolls that have accumulated 165.2 through 672 heat units beyond anthesis (ca. 7 to 27 d) are more frequently injured by brown stink bug when a range of boll ages are available. The susceptible boll ages in these studies corresponded to a boll diameter of 1.161-3.586 cm with a mid range of 2.375 cm. Sampling bolls within this defined range (approximately the size of a United States quarter), that are likely to exhibit injury, should improve the ability of this method to detect stink bug infestations in cotton.

During each week in 2002 and 2003, significantly more bolls with ≥ 1 injured carpel (≤ 7.5-fold), bolls with ≥ 2 injured carpels (≤ 15.0-fold), and bolls exhibiting lint discoloration (≤ 8.6-fold), were recorded in the stink bug-infested treatment compared to that in the non-infested treatment. Significantly fewer bolls displayed internal carpel injury and in combination with external boll wall symptomology, as compared to bolls with only internal carpel injury. No significant differences were observed between bolls

with internal lint discoloration and carpel injury, and bolls with only internal carpel injury without lint discoloration. External symptoms significantly underestimate stink bug-injured bolls in a cotton field. Therefore, sampling methodologies that rely on opening bolls for internal boll wall, lint, and seed injury, rather than examining the outer surface of bolls for external symptoms, should be a stronger indicator of stink bug presence in a cotton field.

Total boll density across all cohorts increased 6.6 and 5.1-fold from week one to week five in 2002 and 2003, respectively. There was a corresponding 6.2-fold and 4.6-fold increase in 2002 and 2003, respectively, for total bolls injured from week one to week five. A positive relationship was observed between injured bolls and boll density across weeks. However, the rate at which brown stink bug injured bolls was much less than the rate at which cotton plants developed bolls. Percent boll injury ranged from 10.7 (week 4) to 27.4 (week 2) in 2002 and 9.2 (week 3) to 16.0 (week 2) in 2003. Percent injury was greatest during weeks one and two in both years and in week five in 2002. Generally, percent injury was greatest during weeks in which boll density was lowest because the ratio of total bolls to injured bolls was much lower compared to weeks in which boll density was high. Therefore, action thresholds that rely on percent injury should consider the time of season because cotton plant phenology and boll density are dynamic factors. More bolls should be examined in the current sampling protocol to accurately classify the percent boll injury for a cotton field if a constant density of stink bugs are present. The level of stink bugs present during each week in our studies was 8.2-fold greater than a current action threshold (one stink bug / six row-ft).

Brown stink bug significantly reduced seedcotton yield of bolls present on cotton plants during weeks one, two, and five in 2002 and in weeks four and five in 2003. However, total seedcotton yield (bolls exposed to brown stink bug and bolls set on plants following infestations) was not significantly reduced for infestations that occurred during weeks one through four in 2002 and weeks one through three in 2003. Cotton plants compensated for stink bug injury occurring during the initial periods of flowering. In our studies, compensation was facilitated because optimal growing conditions were present and other pests were managed appropriately. Infestations that occurred during the final 7 to 14 d of the flowering period reduced seedcotton yield because of the abbreviated period for the cotton plant to compensate for boll injury that occurred at the end of the flowering season. In our studies, stink bug injury was measured on bolls that remained on cotton plants following weekly infestations. However, boll injury may also have been manifested as loss of an entire boll because stink bug feeding can induce abscission in small bolls. Compensation in our studies likely occurred as production of more bolls or greater seedcotton weights in individual bolls.

Managing stink bug infestations that exceed treatment thresholds during flowering will reduce seedcotton yield losses that directly result from feeding. However, infestations that occur beyond flowering and into phenological stages of boll maturation and opening, will also influence other harvest losses (boll rots and hard locked carpels). Studies were conducted to determine how southern green stink bug can interact with environmental conditions conducive for the development of plant pathogens to indirectly influence other harvest losses in cotton. Stink bug feeding wounds on bolls have been suggested as a means of entry for the invasion of bacterial and fungal pathogens.

Stink bug-infested and non-infested cotton plants were exposed to natural or simulated rainfall (2.5 to 3.75 cm) for a five-week period. The percentage of rotted (2.0-fold) and hard locked (1.4-fold) bolls within the southern green stink bug-infested treatment was significantly greater compared to that in the non-infested treatment. Stink bug injury within hard locked (1.9-fold) and harvestable (1.7-fold) bolls was more common in the infested treatment compared to those bolls in the non-infested treatment. Stink bug injury was observed in harvestable (20.3%) and hard locked (35.8%) bolls. Therefore, other abiotic and/or biotic factors likely contribute to late-season harvest losses in cotton. Also, stink bug injury in developing bolls does not always result in a harvest loss when persistent rainfall and humidity are present. In our studies, stink bug injury was present in harvestable bolls. Stink bugs significantly reduced the proportion of harvestable bolls as well as the amount of seedcotton, lint, and seed yield in the infested treatment compared to the non-infested treatment. Significantly more (1.5-fold) seedcotton from hard locked bolls was detected in the stink bug-infested treatment. Stink bugs reduced germination of seed from harvestable bolls (1.2-fold), but germination from hard locked bolls was not different between treatments. Southern green stink bug feeding can be associated with a higher incidence of rotted and hard locked bolls when conditions of high rainfall and humidity occur in cotton fields.

These studies have demonstrated stink bugs can directly injure bolls by increasing boll abscission rates and reducing individual boll weights or indirectly through boll rots and hard locked carpels. Both types of injury resulted in subsequent yield losses. Application of an effective insecticide will be a critical component in the management of threshold levels of stink bugs in cotton. Field observations and historical data suggest

insecticide susceptibility varies among stink bug species and for specific life stages within a given species. Currently, comprehensive data is not available that describes the susceptibility of stink bugs to new insecticide chemistries, including the pyrethroids. Studies evaluated the susceptibility of common stink bug species and life stages to insecticides commonly used for management of stink bugs.

The adult vial test (AVT) was used to test organophosphate and pyrethroid insecticides against adults and/or nymphs of brown stink bug; *E. quadrator* Rolston, another brown colored species; southern green stink bug; and green stink bug. Acephate was more toxic than dicrotophos to brown stink bug adults. Brown stink bug and southern green stink bug adults were equally sensitive to dicrotophos. Generally, brown stink bug adults were most sensitive to the pyrethroid, bifenthrin (1.8 to 6.5-fold), compared to other pyrethroids. Brown stink bug adults were significantly less susceptible than southern green stink bug adults to cyfluthrin (3.9-fold), cypermethrin (2.9 to 33.8-fold), and λ-cyhalothrin (7.6 to 66.5-fold). The AVT LC_{50}'s (μg/vial) for pyrethroids ranged from 0.27 to 2.55, 0.06 to 0.40, and 0.02 to 0.58 for brown stink bug adults, late-instar nymphs (of all species), and southern green stink bug adults, respectively. The order of susceptibility of stink bug species and development stages to insecticides from least to most susceptible was adult *Euschistus* spp. < late-instar nymphs < southern green stink bug adults. In field studies, acephate, dicrotophos, and high rates of bifenthrin, cypermethrin, cyfluthrin, *z*-cypermethrin, and λ-cyhalothrin-treated plant tissue produced significant levels of brown stink bug adult mortality (52.5 to 89.2%) compared to non-treated controls ($P < 0.01$).

These results have established base-line mortality data of several insecticides for use in future monitoring programs. These data also provide evidence that stink bug species and life stages are not equally susceptible to insecticides. Implications for these results support the need to properly identify stink bugs species before choosing an insecticide.

Integrated pest management (IPM) plans for any insect pest of a crop are based upon an understanding of crop injury, implementation of an effective sampling plan and action thresholds, and having an awareness of available and effective control measures for the pest. Results from these studies will be incorporated into each of these IPM components in an effort to refine management programs for stink bugs across the mid-southern and southeastern Unites States. This is particularly noteworthy because stink bugs have become more common in the absence of boll weevils and utilization of transgenic (*Bt*) cultivars with no efficacy against hemipteran pests. These comprehensive studies have defined stink bugs to be significant pests of cotton during growth stages when bolls are available. During these phenological stages of cotton, sampling for stink bug injury in bolls should be intensified. Sampling bolls with our defined range (165 through 672 heat units beyond anthesis) will likely increase a crop manager's ability to detect the presence of a stink bug infestation in a cotton field and to make an appropriate management decision. Insecticide treatments targeted against a stink bug complex should consider species and life stage because brown stink bug adults are less susceptible than other species and life stages to insecticides.

APPENDIX A

LETTER OF PERMISSION AND DATA FOR CHAPTER 2

Letter of permission from the Journal of Economic Entomology to reprint Chapter 2 and data from no-choice studies which measured boll abscission (Figure 2.2), seedcotton yield (Figure 2.3), proportion hard locked carpels (Figure 2.4), seed germination (Figure 2.5), and boll growth (Figure 2.6, 2.7), as influenced by brown stink bug adults.

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The sender of this message has requested a read receipt. Click here to send a receipt.

Willrich, Melissa

From: Alan Kahan [akahan@entsoc.org] **Sent:** Sat 4/3/2004 9:47 AM
To: Willrich, Melissa
Cc:
Subject: Fw: letter of permission to reprint
Attachments:

April 3, 2004

Ms. Melissa Willrich
Louisiana State University
Department of Entomology
402 Life Sciences Building
Baton Rouge, LA 70803

Dear Ms. Willrich,

The Entomological Society of America grants you permission to use the article cited below in a chapter of your dissertation for Louisiana State University.

Willrich, M., L. B. Rogers, and J. Temple. 2004. Injury to Pre-flowering and Flowering Cotton, *Gossypium hirsutum* (L.), by Brown Stink Bug, *Euschistus servus* (Say), and Southern Green Stink Bug, *Nezara viridula* (L.). Journal of Economic Entomology. *In press.*

Sincerely,

Alan Kahan
Director of Communications
Entomological Society of America
10001 Derekwood Lane, Suite 100
Lanham, MD 20706-4876
Phone: 301-731-4535, ext.3020
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akahan@entsoc.org

----- Original Message -----
From: Willrich, Melissa
To: Alan Kahan
Sent: Tuesday, March 30, 2004 7:23 AM
Subject: RE: letter of permission to reprint

Hello Alan,

The following article, "Injury to Pre-flowering and Flowering Cotton, *Gossypium hirsutum* (L.), by Brown Stink Bug, *Euschistus servus* (Say), and Southern Green Stink Bug, *Nezara viridula* (L.)", has been accepted for publication in the Journal of Economic Entomology (EC-03-282) and is currently at the printing department. The data from this manuscript will also be published as a chapter of my dissertation from Louisiana State University. This dissertation will be submitted to the graduate school this coming June. Could I request a letter stating that ESA and the Journal of Economic Entomology give me permission to reprint the data from this manuscript for my dissertation?

The letter can be addressed to me, Melissa Willrich, at the following address: Louisiana State University, Department of Entomology, 402 Life Sciences Building, Baton Rouge, LA 70803.

Thanks,
Melissa Willrich

Table A.1. Brown stink bug-induced boll abscission for boll of various ages (heat units accumulated beyond anthesis).

Boll Age (heat units)	Boll Abscission (%)
0-50	28.6
51-100	50.9
101-150	32.6
151-200	25.0
201-250	2.0
251-300	2.8
301-350	11.8
351-400	0.0
401-450	0.0
451-500	0.0
501-550	0.0
551-600	0.0
601-650	0.0
651-700	0.0
701-750	0.0
751-800	-----
801-850	0.0
851-900	0.0

Table A.2. Seedcotton weights for bolls of various ages (heat units accumulated beyond anthesis) infested with brown stink bug or non-infested.

Boll Age (heat units)	Treatment	Boll Weight (g)
0-50	Infested	3.735
	Non-infested	4.393
51-100	Infested	2.123
	Non-infested	4.193
101-150	Infested	2.878
	Non-infested	4.154
151-200	Infested	2.649
	Non-infested	4.250
201-250	Infested	2.997
	Non-infested	4.347
251-300	Infested	3.261
	Non-infested	4.164
301-350	Infested	3.350
	Non-infested	3.955
351-400	Infested	3.145
	Non-infested	4.421
401-450	Infested	3.649
	Non-infested	4.134
451-500	Infested	3.913
	Non-infested	4.554
501-550	Infested	4.016
	Non-infested	4.352
551-600	Infested	4.113
	Non-infested	4.020
601-650	Infested	4.196

Table A.2. Continued.

	Non-infested	4.148
651-700	Infested	4.202
	Non-infested	4.316
701-750	Infested	3.900
	Non-infested	4.206
751-800	Infested	-----
	Non-infested	-----
801-850	Infested	4.359
	Non-infested	4.455
851-900	Infested	3.371
	Non-infested	3.855

Table A.3. Proportion of hard locked carpels for bolls of various ages (heat units accumulated beyond anthesis) infested with brown stink bug or non-infested.

Boll Age (heat units)	Treatment	Proportion Hard Locked Carpels
0-50	Infested	0.24
	Non-infested	0.16
51-100	Infested	0.29
	Non-infested	0.06
101-150	Infested	0.40
	Non-infested	0.21
151-200	Infested	0.53
	Non-infested	0.19
201-250	Infested	0.69
	Non-infested	0.20
251-300	Infested	0.57
	Non-infested	0.20
301-350	Infested	0.47
	Non-infested	0.18
351-400	Infested	0.44
	Non-infested	0.18
401-450	Infested	0.26
	Non-infested	0.18
451-500	Infested	0.25
	Non-infested	0.11
501-550	Infested	0.10
	Non-infested	0.02
551-600	Infested	0.40
	Non-infested	0.32
601-650	Infested	0.35
	Non-infested	0.11
651-700	Infested	0.25
	Non-infested	0.21
701-750	Infested	-----
	Non-infested	-----
751-800	Infested	-----
	Non-infested	-----
801-850	Infested	0.10
	Non-infested	0.15
851-900	Infested	-----
	Non-infested	-----

Table A.4. Seed germination from bolls of various ages (heat units accumulated beyond anthesis) infested with brown stink bug or non-infested.

Boll Age (heat units)	Treatment	Germination (%)
0-100	Infested	58.9
	Non-infested	57.4
101-200	Infested	39.9
	Non-infested	51.8
201-300	Infested	31.4
	Non-infested	42.9
301-400	Infested	23.2
	Non-infested	47.5
401-500	Infested	37.7
	Non-infested	53.0
501-600	Infested	44.0
	Non-infested	59.0
601-700	Infested	41.4
	Non-infested	40.6
701-800	Infested	13.9
	Non-infested	28.0
801-900	Infested	29.7
	Non-infested	41.9

Table A.5. Diameters for bolls of various ages (heat units accumulated beyond anthesis) infested with brown stink bug , non-infested, or non-caged. Data were used to define the quadratic relationship between boll age and diameter.

Boll Age (heat units)	Treatment	Diameter (cm)
67.5	Infested	1.275
	Non-infested	1.325
	Non-caged	-----
74.1	Infested	-----
	Non-infested	-----
	Non-caged	1.085
78.0	Infested	1.093
	Non-infested	1.268
	Non-caged	1.090
81.5	Infested	-----
	Non-infested	-----
	Non-caged	1.130
82.5	Infested	-----
	Non-infested	-----
	Non-caged	1.100
93.5	Infested	1.160
	Non-infested	1.230
	Non-caged	-----
107.0	Infested	1.383
	Non-infested	1.513
	Non-caged	-----
116.5	Infested	1.305
	Non-infested	1.475

Table A.5. Continued.

	Non-caged	-----
123.5	Infested	-----
	Non-infested	-----
	Non-caged	1.440
133.5	Infested	-----
	Non-infested	-----
	Non-caged	1.550
137.0	Infested	-----
	Non-infested	-----
	Non-caged	1.460
156.0	Infested	1.488
	Non-infested	1.865
	Non-caged	-----
171.0	Infested	1.675
	Non-infested	2.065
	Non-caged	-----
172.9	Infested	-----
	Non-infested	-----
	Non-caged	1.880
180.0	Infested	-----
	Non-infested	-----
	Non-caged	1.800
184.5	Infested	-----
	Non-infested	-----
	Non-caged	1.900
185.0	Infested	2.070
	Non-infested	2.255
	Non-caged	-----
208.0	Infested	1.895
	Non-infested	2.248
	Non-caged	-----
214.0	Infested	2.173
	Non-infested	2.325
	Non-caged	-----
222.3	Infested	-----
	Non-infested	-----
	Non-caged	2.280
229.0	Infested	-----
	Non-infested	-----
	Non-caged	2.400
231.0	Infested	-----
	Non-infested	-----
	Non-caged	2.380
233.0	Infested	-----
	Non-infested	-----
	Non-caged	2.300
234.5	Infested	2.445
	Non-infested	2.730
	Non-caged	-----
242.0	Infested	2.573
	Non-infested	2.690
	Non-caged	-----
256.9	Infested	2.783

Table A.5. Continued.

	Non-infested	2.908
	Non-caged	-----
266.5	Infested	2.600
	Non-infested	2.793
	Non-caged	-----
271.7	Infested	-----
	Non-infested	-----
	Non-caged	2.700
280.0	Infested	-----
	Non-infested	-----
	Non-caged	2.800
285.0	Infested	3.020
	Non-infested	3.058
	Non-caged	-----
310.0	Infested	2.923
	Non-infested	2.980
	Non-caged	-----
321.1	Infested	-----
	Non-infested	-----
	Non-caged	3.190
331.0	Infested	-----
	Non-infested	-----
	Non-caged	3.220
333.5	Infested	2.945
	Non-infested	3.055
	Non-caged	2.910
357.5	Infested	2.978
	Non-infested	3.210
	Non-caged	-----
370.5	Infested	-----
	Non-infested	-----
	Non-caged	3.250
376.0	Infested	-----
	Non-infested	-----
	Non-caged	3.200
383.0	Infested	-----
	Non-infested	-----
	Non-caged	3.010
401.0	Infested	3.358
	Non-infested	3.220
	Non-caged	-----
404.5	Infested	-----
	Non-infested	-----
	Non-caged	3.410
418.0	Infested	3.198
	Non-infested	3.178
	Non-caged	-----
419.9	Infested	-----
	Non-infested	-----
	Non-caged	3.330
421.5	Infested	-----
	Non-infested	-----
	Non-caged	3.300
432.0	Infested	-----

Table A.5. Continued.

	Non-infested	-----
	Non-caged	3.140
450.0	Infested	-----
	Non-infested	-----
	Non-caged	3.500
462.5	Infested	3.330
	Non-infested	3.325
	Non-caged	-----
467.5	Infested	-----
	Non-infested	-----
	Non-caged	3.300
469.3	Infested	-----
	Non-infested	-----
	Non-caged	3.280
474.5	Infested	3.373
	Non-infested	3.300
	Non-caged	-----
477.5	Infested	-----
	Non-infested	-----
	Non-caged	3.200
489.5	Infested	3.193
	Non-infested	3.195
	Non-caged	-----
496.0	Infested	-----
	Non-infested	-----
	Non-caged	3.500
511.5	Infested	-----
	Non-infested	-----
	Non-caged	3.300
518.4	Infested	-----
	Non-infested	-----
	Non-caged	3.240
523.0	Infested	-----
	Non-infested	-----
	Non-caged	3.200
540.5	Infested	3.265
	Non-infested	3.320
	Non-caged	3.400
552.0	Infested	-----
	Non-infested	-----
	Non-caged	3.300
557.9	Infested	-----
	Non-infested	-----
	Non-caged	3.130
562.5	Infested	-----
	Non-infested	-----
	Non-caged	3.100
559.5	Infested	3.303
	Non-infested	3.340
	Non-caged	-----
580.5	Infested	-----
	Non-infested	-----
	Non-caged	3.320
582.0	Infested	3.338

Table A.5. Continued.

	Non-infested	3.293
	Non-caged	-----
585.0	Infested	3.258
	Non-infested	3.293
	Non-caged	-----
592.0	Infested	3.215
	Non-infested	3.288
	Non-caged	-----
651.5	Infested	3.250
	Non-infested	3.313
	Non-caged	-----
685.5	Infested	3.263
	Non-infested	3.323
	Non-caged	-----
729.0	Infested	3.331
	Non-infested	3.380
	Non-caged	-----
891.5	Infested	3.243
	Non-infested	3.260
	Non-caged	-----

Table A.6. Diameters for bolls of various ages (heat units accumulated beyond anthesis) infested with brown stink bug or non-infested. Data were used to compare treatments within boll ages.

Boll Age (heat units)	Treatment	Diameter (cm)
67.5	Infested	1.275
	Non-infested	1.325
78.0	Infested	1.093
	Non-infested	1.268
93.5	Infested	1.160
	Non-infested	1.230
107.0	Infested	1.383
	Non-infested	1.513
116.5	Infested	1.305
	Non-infested	1.475
156.0	Infested	1.488
	Non-infested	1.865
171.0	Infested	1.675
	Non-infested	2.065
185.0	Infested	2.070
	Non-infested	2.255
208.0	Infested	1.895
	Non-infested	2.248
214.0	Infested	2.173
	Non-infested	2.325
234.5	Infested	2.445
	Non-infested	2.730
242.0	Infested	2.573
	Non-infested	2.690
256.9	Infested	2.783
	Non-infested	2.908

Table A.6. Continued.

266.5	Infested	2.600
	Non-infested	2.793
285.0	Infested	3.020
	Non-infested	3.058
310.0	Infested	2.923
	Non-infested	2.980
333.5	Infested	2.945
	Non-infested	3.055
357.5	Infested	2.978
	Non-infested	3.210
401.0	Infested	3.358
	Non-infested	3.220
418.0	Infested	3.198
	Non-infested	3.178
462.5	Infested	3.330
	Non-infested	3.325
474.5	Infested	3.373
	Non-infested	3.300
489.5	Infested	3.193
	Non-infested	3.195
540.5	Infested	3.265
	Non-infested	3.320
559.5	Infested	3.303
	Non-infested	3.340
582.0	Infested	3.338
	Non-infested	3.293
585.0	Infested	3.258
	Non-infested	3.293
592.0	Infested	3.215
	Non-infested	3.288
651.5	Infested	3.250
	Non-infested	3.313
685.5	Infested	3.263
	Non-infested	3.323
729.0	Infested	3.331
	Non-infested	3.380
891.5	Infested	3.243
	Non-infested	3.260

APPENDIX B

DATA FOR CHAPTER 3

Data from Chapter 3 which measured total bolls, total bolls injured, and percent injury within the initial five weeks of flowering, 2002 and 2003 (Figure 3.2).

Table B.1. Total bolls, total bolls injured, and percent boll injury, 2002 and 2003.

	2002			2003		
Week	Total Bolls	Total Bolls Injured	Injury (%)	Total Bolls	Total Bolls Injured	Injury (%)
1	47.3	10.4	22.0	67.8	8.7	12.9
2	103.0	28.2	27.4	137.5	22.0	16.0
3	254.5	34.6	13.6	233.5	21.5	9.2
4	296.5	41.7	10.7	241.3	29.2	11.6
5	311.0	64.4	20.7	348.0	40.0	11.5

APPENDIX C

DATA FOR CHAPTER 4

Data from whole-plant infestations which measured the proportion of boll cohorts per week (Figure 4.1, 4.2), boll injury among cohorts within weeks (Figure 4.1, 4.2), boll injury among cohorts across weeks (Figure 4.3), and boll diameter among cohorts across weeks (Figure 4.3), 2002 and 2003.

Table C.1. Mean number of bolls per cohort (heat units accumulated) within a week, proportion of bolls per cohort of total boll present within a week, and percent of bolls within each cohort with injury by brown stink bug, 2002.

Week	Boll Cohort (heat units accumulated)	Mean Number Bolls	Percent of Total Bolls	Percent Injury
1	1 (<165.2)	30.9	76.9	13.7
1	2 (165.3-330.5)	9.3	23.1	40.4
2	1 (<165.2)	50.6	62.2	4.9
2	2 (165.3-330.5)	21.5	26.4	53.8
2	3 (330.6-495.8)	9.3	11.4	60.2
3	1 (<165.2)	78.1	42.6	0.6
3	2 (165.3-330.5)	51.0	27.8	15.3
3	3 (330.6-495.8)	41.8	22.8	21.0
3	4 (495.9-661.1)	12.4	6.8	27.9
4	1 (<165.2)	12.0	7.3	9.6
4	2 (165.3-330.5)	47.5	28.7	12.5
4	3 (330.6-495.8)	60.3	36.5	17.4
4	4 (495.9-661.1)	34.3	20.7	13.3
4	5 (661.2-826.4)	11.3	6.8	15.5
5	1 (<165.2)	8.0	4.0	0.0
5	2 (165.3-330.5)	32.6	16.3	32.8
5	3 (330.6-495.8)	61.9	30.9	29.8
5	4 (495.9-661.1)	52.5	26.2	12.3
5	5 (661.2-826.4)	36.8	18.4	11.3
5	6 (826.5-850.5)	8.6	4.3	5.0

Table C.2. Mean number of bolls per cohort (heat units accumulated) within a week, proportion of bolls per cohort of total boll present within a week, and percent of bolls within each cohort with injury by brown stink bug, 2003.

Week	Boll Cohort (heat units accumulated)	Mean Number Bolls	Percent of Total Bolls	Percent Injury
1	1 (<168)	37.0	56.4	0.0
1	2 (169-336)	23.0	35.1	21.9
1	3 (337-504)	5.5	8.4	44.2
2	1 (<168)	56.3	41.5	0.9
2	2 (169-336)	49.8	36.8	24.7
2	3 (337-504)	26.5	19.6	36.5
2	4 (505-672)	2.6	1.9	66.5
3	1 (<168)	84.6	35.8	0.0
3	2 (169-336)	84.0	35.6	18.2
3	3 (337-504)	44.8	19.0	11.8
3	4 (505-672)	20.4	8.6	7.0
3	5 (673-840)	2.2	0.9	0.0

Table C.2. Continued.

4	1 (<168)	32.1	13.9	0.0
4	2 (169-336)	44.9	19.5	19.8
4	3 (337-504)	86.9	37.7	15.6
4	4 (505-672)	47.3	20.5	8.2
4	5 (673-840)	16.6	7.2	14.7
4	6 (841-1014)	2.5	1.1	0.0
5	1 (<168)	40.8	13.0	3.6
5	2 (169-336)	21.5	6.8	13.4
5	3 (337-504)	83.0	26.4	14.2
5	4 (505-672)	92.3	29.4	11.4
5	5 (673-840)	53.5	17.0	8.8
5	6 (841-1014)	23.1	7.4	11.6

Table C.3. Mean diameter and injury among cohorts across weeks, 2002 and 2003.

	2002		2003	
Boll Cohort	Diameter (cm)	Injury (%)	Diameter (cm)	Injury (%)
1	1.257	8.8	1.160	0.8
2	2.329	30.9	2.240	19.6
3	2.807	32.1	2.809	24.4
4	2.918	17.8	2.929	16.1
5	2.990	13.4	2.978	8.5
6	2.900	5.0	2.927	6.6

APPENDIX D

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Journal of Cotton Science

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Published by the Cotton Foundation

January 6, 2004

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JCS-rrm 1/6/04

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